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## EFFECT OF LIME UPON THE SODIUM-CHLORID TOLERANCE OF WHEAT SEEDLINGS

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### INTRODUCTION

The work reported here is a continuation of that previously reported in Bulletin 149 of the Bureau of Chemistry. In this former work the beneficial effect of lime upon soils which have become acid by the continued use of potassium chlorid or potassium sulphate was pointed out. The present work takes up an equally important rôle of lime—its effect upon some of the salts commonly occurring in "alkali" soils.

The concentration of nutrient salts best suited to plant growth<sup>2</sup> is entirely different in sand and in solution cultures. Seedlings grown in a solution of 2,500 parts per million of nutrient salts suffer, while those grown in sand and watered continuously with the solution, with free drainage, produce vigorous plants. From this it appears that inert material, such as sand, clay, and soil, might materially affect the toxic limit of dissolved salts. It was, therefore, desired to see whether a plant growing in a stiff soil would be more resistant to sodium chlorid, which is one of the salts found in alkali soils, than one growing in a sandy soil or in a solution.

### EXPERIMENTS

#### SERIES I

Several small glass jars having a capacity of 250 cc. were filled with sodium-chlorid solutions of the following concentrations:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	Distilled water.
2	500 parts per million of sodium chlorid.
3	1,000 parts per million of sodium chlorid.
4	2,000 parts per million of sodium chlorid.
5	3,000 parts per million of sodium chlorid.
6	4,000 parts per million of sodium chlorid.

<sup>1</sup> Much appreciation on the part of the authors is due to Dr. J. Davidson for his painstaking review and correction of the manuscript.

<sup>2</sup> BREAZEALE, J. F. EFFECT OF THE CONCENTRATION OF THE NUTRIENT SOLUTION UPON WHEAT CULTURES. *In Science*, V. 22, no. 553, p. 146-149. 1905.

Six wheat seedlings were introduced into these jars and held in place through slits in the corks by rubber bands. The seedlings had been sprouted in pans of distilled water on floating perforated aluminum disks and allowed to grow for three days, or until they were about 10 cm. long, before being placed in the solutions. At the same time a similar set of jars, with small holes drilled in the bottom for drainage, were filled with quartz sand and planted with wheat seed. These sand cultures were watered almost continuously during the day with sodium-chlorid solution of the same strength as that used in the corresponding water culture. By forcing a large amount of solution through each jar the concentration of the solution in the sand was kept fairly constant. This was frequently checked by titrating the solution before and after it passed through the sand.

When the plants were 8 days old they were removed and photographed. The plants grown in solution are shown in Plate 38, A, and those grown in sand in Plate 38, B. It will be seen from Plate 38, A, that the toxic action of sodium chlorid manifests itself, especially upon the root development, in all the concentrations tried. This was not true in the sand culture, in which an increase in growth was noticed up to a concentration of 1,000 parts per million. In comparing the two illustrations it will be noticed also that the higher concentrations were more toxic in the sand than in the solution cultures. This was probably due to the fact that sand cultures were started from the seed, while the solution cultures were started from seedlings 3 days old, the plant being apparently more sensitive to sodium chlorid during the first few days of its growth.

This necessitated a change in the method of sprouting the seedlings. In all the experiments hereafter to be described the seedlings were sprouted in the same concentration of the solution as that in which they were afterwards to be placed.

#### SERIES 2

The next experiment was begun on February 26, with the following solutions:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	Distilled water.
2	500 parts per million of sodium chlorid.
3	1,000 parts per million of sodium chlorid.
4	1,500 parts per million of sodium chlorid.
5	2,000 parts per million of sodium chlorid.
6	3,000 parts per million of sodium chlorid.
7	4,000 parts per million of sodium chlorid.

Large enameled pans holding about 3 liters were filled with the solutions of the different concentrations, and perforated aluminum disks were floated in them. Wheat seeds were sprouted upon the disks.

As soon as the radicles appeared, usually at the end of 24 hours, the seeds for the sand or soil cultures were taken out and planted. The pans were allowed to stand until the shoots of the other seeds had reached a height of about 1 cm., when they were transferred to the culture bottles. In addition to the sand and solution cultures, this experiment included a series of bottles filled with very fine soil—a volcanic ash obtained from Jamaica, consisting of about 98 per cent of iron and aluminum oxid, principally iron. The bottles of soil and sand were watered with these solutions before planting until the drainage titrated the same for chlorid as did the original solutions. This took about 2 days in the soil cultures, since only about 250 cc. of the solution moved through the clay in an hour. With the sand it took a much shorter time.

On March 5, when the plants were 8 days old, they were photographed (Pl. 39).

As in Plate 38 it will be seen that the toxic action of the sodium chlorid was first noticeable in the solution cultures, even in the 500 parts per million solution, the lowest concentration; and the effect was more and more appreciable, especially upon the root development, as the concentration increased. It was not noticeable in the sand cultures in the lower concentrations, in which the root development showed no marked difference until a concentration of 2,000 parts per million was reached. With the soil cultures, no toxic effect of the sodium chlorid on the growth of the plants could be seen, even in the highest concentration. In fact, the plants growing in the soil and watered almost continuously with a 4,000 parts per million sodium-chlorid solution grew as well as, if not better than, those growing in the same soil and watered with distilled water. The only noticeable effect of the sodium chlorid upon the soil cultures was the tendency to cause lodging, especially as the plants grew older, this tendency increasing with the amount of sodium chlorid. This is shown in Plate 40, A, which shows the plants when 9 days old.

#### SERIES 3

On March 12 another set of solution, sand, and soil pots was started, with concentrations of sodium chlorid running from 1,000 to 4,000 parts per million. On March 15 one plant was withdrawn from each group where the highest concentration was used and a photograph was made. The 3-day-old seedlings which had been grown in 4,000 parts per million solution (Pl. 40, B) were so small that they were still dependent upon the seed and had not yet begun to feed upon the solution to any appreciable extent. It was evident that the effect of the soil was manifested upon the young plant at a very early stage in its life history.

## SERIES 4

To test the effect of the practically inert material represented by the soil, two pots were started, one in a 4,000 parts per million solution of sodium chlorid and the other in pure carbon black, watered with 4,000 parts per million sodium-chlorid solution. The young plants grown in carbon black watered with 4,000 parts per million sodium chlorid were just as poor as those grown in 4,000 parts per million sodium-chlorid solution, indicating that the effect of the soil in overcoming the toxicity of sodium chlorid was due merely to the presence of inert particles.

## SERIES 5

On March 6 a set of soil bottles was prepared, arranged on a filter rack so that the drainage could be easily collected, and watered with the following solutions:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	Distilled water.
2	1,000 parts per million of sodium chlorid.
3	1,500 parts per million of sodium chlorid.
4	2,000 parts per million of sodium chlorid.
5	3,000 parts per million of sodium chlorid.
6	4,000 parts per million of sodium chlorid.

When it was found by titration that the drainage was of the same salt content as the original solution, 200 cc. that had passed through the soil and the sand, respectively, were collected from under each pot. These solutions were put into culture bottles and, together with the sand and soil pots, planted with wheat seedlings in the manner before described. We now had plants growing in sand, in the solution that had passed through the sand, in soil, and in the solution that had passed through the soil. The sand and soil pots were watered frequently during the day with their respective solutions until the seedlings were 7 days old, when they were photographed (Pl. 41).

The plants grown in the sand pots differed very little from those grown in the solutions that had passed through the sand (Pl. 41, A). The limit of tolerance for each was 3,000 parts per million of sodium chlorid. No difference in the plants growing in the soil could be detected, even in the highest concentration (Pl. 41, B). Although none of the plants grown in the solution which had passed through the soil were quite so good as the control, excellent plants were obtained even in the highest concentration—4,000 parts per million, which is considerably above the limit of tolerance of sodium chlorid as shown in sand.

The plants were allowed to grow until March 16, or until they were 10 days old. They were then uprooted or removed from the solution and photographed. The plants growing in the sand are shown in Plate 42, A, and those grown in soil in Plate 42, B. The plants grown in the solutions

which had passed through the sand and likewise in the solutions which had passed through the soil were similar to those grown in the sand and soil. It is evident, therefore, that in passing through the soil the solution was so altered as to overcome almost completely the toxic effect of the sodium chlorid.

#### SERIES 6

On March 13 another set was started with the following solutions:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	4,000 parts per million of sodium chlorid.
2	6,000 parts per million of sodium chlorid filtered through the soil.
3	8,000 parts per million of sodium chlorid filtered through the soil.
4	4,000 parts per million of sodium chlorid filtered through the soil.
5	4,000 parts per million of sodium chlorid added to distilled water that had previously been filtered through the soil.
6	4,000 parts per million of sodium chlorid + 100 parts per million each of sodium nitrate, potassium chlorid, and sodium phosphate.

On March 20 the plants were photographed (Pl. 43, A). It will be seen that vigorous plants were obtained in solution 3 in a concentration of 8,000 parts per million sodium chlorid, or double the amount necessary to stop the growth in distilled water, by simply filtering the salt solution through the soil. Good plants were also obtained in solution 5 to which the salt had been added after the distilled water had been passed through the soil. In solution 6 the addition of the fertilizer salts produced comparatively little better growth than was noted in the control.

Two hundred cc. of water were then passed through a fresh pot of soil and analyzed. The salt content of the solution showed 31 parts per million of calcium oxid. This suggested the possibility that lime might be the cause of the greater tolerance noticed in all the soil pots.

#### SERIES 7

To test the effect of lime the following set was started:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	4,000 parts per million of sodium chlorid.
2	4,000 parts per million of sodium chlorid + 30 parts per million of calcium sulphate.
3	4,000 parts per million of sodium chlorid + 30 parts per million of calcium oxid.
4	4,000 parts per million of sodium chlorid + 30 parts per million of magnesium bicarbonate.

On March 29 the plants were photographed (Pl. 43, B). The magnesium salt had little effect, but the calcium sulphate and calcium oxid were about equally effective in overcoming the toxic action of sodium chlorid.

## SERIES 8

To determine the relation of some other salts to the sodium-chlorid tolerance the following set was started on March 30:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	Distilled water.
2	4,000 parts per million of sodium chlorid + 30 parts per million of calcium sulphate.
3	4,000 parts per million of sodium chlorid + 30 parts per million of calcium oxid.
4	4,000 parts per million of sodium chlorid + 30 parts per million of magnesium sulphate.
5	4,000 parts per million of sodium chlorid + 30 parts per million of barium chlorid.
6	4,000 parts per million of sodium chlorid + 30 parts per million of potassium chlorid.
7	4,000 parts per million of sodium chlorid + 30 parts per million of sodium nitrate.
8	4,000 parts per million of sodium chlorid + 30 parts per million of sodium phosphate.
9	4,000 parts per million of sodium chlorid + 30 parts per million of ferric chlorid.
10	4,000 parts per million of sodium chlorid + 30 parts per million of potassium alum.

At the end of seven days the plants were photographed (Pl. 44). In this experiment the calcium oxid produced the best plants and calcium sulphate the next best. While the effect of the barium chlorid and magnesium sulphate was not marked, it was evident that they exerted some action upon the sodium chlorid, while the potassium chlorid, sodium nitrate, sodium phosphate, ferric chlorid, and alum produced little or no change.

On March 30 two pots of sand were started. No. 1 was watered with 4,000 parts per million sodium-chlorid solution and the other with the same solution to which had been added 30 parts per million of calcium sulphate. The plants were grown for 16 days and then photographed (Pl. 45, A). The beneficial effect of such a minute amount of lime in sand cultures is clearly shown in this experiment.

## SERIES 9

In Plate 45, B, are shown 3-day-old plants grown in the following solutions of sodium sulphate:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	4,000 parts per million of sodium sulphate.
2	4,000 parts per million of sodium sulphate + 30 parts per million of calcium sulphate.
3	4,000 parts per million of sodium sulphate + 30 parts per million of calcium oxid.
4	4,000 parts per million of sodium sulphate + 30 parts per million of magnesium sulphate.
5	4,000 parts per million of sodium sulphate + 30 parts per million of barium chlorid.

With sodium sulphate the beneficial effects of the calcium salts were just as pronounced as they had been with sodium chlorid, while no benefit resulted from the use of either the barium or magnesium salt.

SERIES 10

Plate 46, A, shows the 3-day-old plants grown in the following solutions:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	Distilled water.
2	4,000 parts per million of sodium sulphate.
3	4,000 parts per million of sodium sulphate + 30 parts per million of potassium chlorid.
4	4,000 parts per million of sodium sulphate + 30 parts per million of sodium nitrate.
5	4,000 parts per million of sodium sulphate + 30 parts per million of ferric oxid.
6	4,000 parts per million of sodium sulphate + 30 parts per million of aluminum oxid.

The oxid of iron and aluminum were here included for the reason that they have shown a marked effect upon slightly acid cultures in previous work with wheat seedlings,<sup>1</sup> but neither these nor the two fertilizers showed any effect.

SERIES 11

Plate 46, B, shows 11-day-old plants which had been grown in the following solutions of sodium bicarbonate:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	2,500 parts per million of sodium bicarbonate.
2	2,500 parts per million of sodium bicarbonate + 30 parts per million of sodium nitrate.
3	2,500 parts per million of sodium bicarbonate + 30 parts per million of potassium chlorid.
4	2,500 parts per million of sodium bicarbonate + 30 parts per million of magnesium sulphate.
5	2,500 parts per million of sodium bicarbonate + 30 parts per million of calcium oxid.

The beneficial effects of the lime are shown here also, although not in so great a degree as with sodium chlorid or sodium sulphate. The experiment with sodium bicarbonate was repeated several times; but while the tops showed distinct effects of the calcium salts, no such marked difference was noticed in the root development.

<sup>1</sup> BRAZEALE, J. F., and LE CLERC, J. A. THE GROWTH OF WHEAT SEEDLINGS AS AFFECTED BY ACID OR ALKALINE CONDITIONS. U. S. Dept. Agr. Bur. Chem. Bul. 149, 18 p., 8 pl. 1912.



## SERIES 12

In the experiments previously conducted, the calcium salts were added at the rate of 30 parts per million. Smaller amounts were now added to determine the minimum amount of lime that could exert an appreciable effect upon plants growing in a toxic salt. Plate 47, A, shows plants grown in the following solutions:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	4,000 parts per million of sodium chlorid.
2	Distilled water.
3	4,000 parts per million of sodium chlorid + 40 parts per million of calcium oxid.
4	4,000 parts per million of sodium chlorid + 30 parts per million of calcium oxid.
5	4,000 parts per million of sodium chlorid + 20 parts per million of calcium oxid.

Forty, 30, and 20 parts per million of lime entirely overcame the depressing action of the sodium chlorid and produced plants equal to those grown in distilled water.

## SERIES 13

In Plate 47, B, are shown plants grown in the following solutions:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	4,000 parts per million of sodium chlorid.
2	4,000 parts per million of sodium chlorid + 15 parts per million of calcium oxid.
3	4,000 parts per million of sodium chlorid + 10 parts per million of calcium oxid.
4	4,000 parts per million of sodium chlorid + 2 parts per million of calcium oxid.
5	4,000 parts per million of sodium chlorid + 1 part per million of calcium oxid.

From Plate 47 it will be seen that the size of the plants decreases gradually as the concentration decreases from 40 parts to 1 part per million of lime. Much better plants were grown in the solutions containing 2 parts and 1 part per million, respectively, than in the solution containing no lime. It is evident that the presence of calcium salts, even in most minute quantities, materially affects the tolerance of young wheat seedlings for sodium chlorid.

## SERIES 14

No satisfactory explanation has yet been given for the effect of calcium salts on the toxic properties of other salts. W. J. V. Osterhout<sup>1</sup> concluded, on the basis of a series of experiments, that entrance of ions of sodium chlorid into the protoplasm was greatly hindered or prevented by

<sup>1</sup> OSTERHOUT, W. J. V. THE PERMEABILITY OF PROTOPLASM TO IONS AND THE THEORY OF ANTAGONISM. *J. N. Science*, N. S. V. 35, No. 890, p. 112-115. 1913.

the presence of a small amount of calcium chlorid and that barium and strontium exerted a similar action. J. Loeb,<sup>1</sup> in his work on the effect of various salts on fishes, concluded similarly that the antagonism of the calcium salts toward certain toxic salts was due to their effect on the permeability of the cell.

Series 14 was introduced to determine the effect of small amounts of lime on the actual absorption of sodium chlorid as shown by the analysis of the ash.

The following solutions were used:

SOLUTION NO.	CONCENTRATION OF SOLUTION.
1	Distilled water.
2	4,000 parts per million of sodium chlorid.
3	4,000 parts per million of sodium chlorid + 30 parts per million of calcium oxid.

When the plants were 8 days old, 100 were withdrawn from each pan and the amount of ash and chlorin estimated. Tables I and II give the results of the analyses.

TABLE I.—Analyses of 100 tops

Concentration of solution.	Ash.	Chlorin, calculated to sodium chlorid.
Distilled water.....	Gm. 0.0462	Gm. Trace.
4,000 p. p. m. of sodium chlorid.....	.0775	0.0320
4,000 p. p. m. of sodium chlorid + 30 p. p. m. of calcium oxid.....	.0621	0.0334
100 whole seeds, ungerminated.....	.0710	Trace.

TABLE II.—Analyses of 100 whole plants

Concentration of solution.	Dry weight.	Chlorin, calculated to sodium chlorid.
Distilled water.....	Gm. 2.077	Gm. Trace.
4,000 p. p. m. of sodium chlorid.....	1.993	0.0553
4,000 p. p. m. of sodium chlorid + 30 p. p. m. of calcium oxid.....	2.352	0.0595

Upon repeating this experiment with three different sets of plants, similar results were obtained each time, showing that there was just as much chlorin in the plants growing in the solution containing lime as in those grown in the solution containing no lime. Within the limits of our experiments, lime is not effective in preventing absorption of sodium chlorid by the plant.

<sup>1</sup> LOEB, JACQUES. ÜBER DIE HEMMUNG DER GIFTWIRKUNG VON NaI, NaN<sub>3</sub>, NaCN, NACN UND ANDEREN NÄTRIUMSALZEN. *In* Biochem. Ztschr., Bd. 43, Heft 3, p. 181-202. 1917.

This same experiment was twice repeated, using sodium sulphate instead of sodium chlorid, with the results shown in Table III.

TABLE III.—*Analyses of 100 whole plants*

Concentration of solution.	Dry weight.	Ash.	Sodium sulphate.
Distilled water.....	Gm. 2. 146	Gm. 0. 0667	Gm. Trace.
4,000 p. p. m. of sodium sulphate.....	2. 460	. 0958	0. 0219
4,000 p. p. m. of sodium sulphate + 30 p. p. m. of calcium oxid.....	2. 550	. 1460	. 0522

The results with sodium sulphate are even more striking than those with sodium chlorid. Instead of hindering the absorption of sodium sulphate by the plant, the calcium oxid actually stimulated it. Even when calculated upon the basis of grams of dry weight, there is still a preponderance of sodium sulphate in the plants grown in the presence of the small amount of lime.

#### CONCLUSIONS

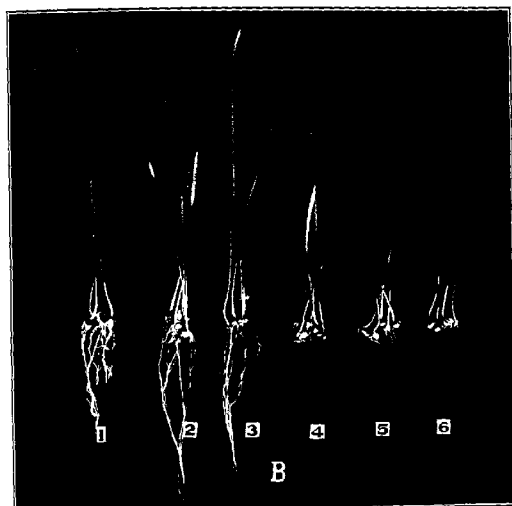
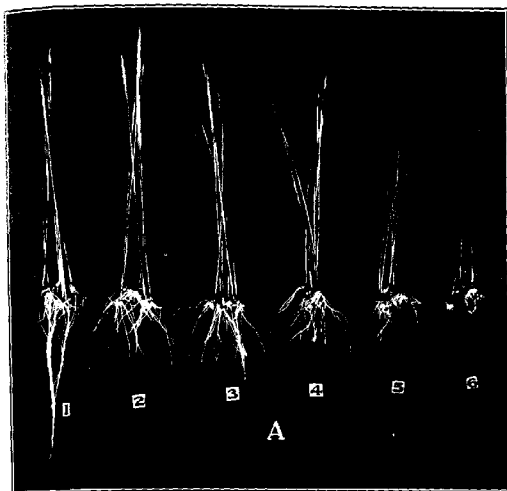
- (1) The higher tolerance to alkali salts shown by plants in soil and sand than by those grown in water cultures is not due entirely to the physical effect of the presence of solid particles of different degrees of fineness, but also to certain soluble substances which are sometimes present in very small quantities.
- (2) Very small amounts of calcium oxid and calcium sulphate overcame the toxic effects of sodium chlorid and sodium sulphate.
- (3) Magnesium sulphate and barium chlorid were slightly antagonistic to sodium chlorid. Potassium chlorid, sodium nitrate, sodium phosphate, ferric chlorid, and alum had no effect on the toxicity of sodium chlorid.
- (4) Within the limits of our experiments the presence of lime did not prevent the entrance of sodium chlorid and sodium sulphate into the plant cells. The antagonistic effects of lime would seem to be due not to its effect on the permeability of the cells but to some other cause.



PLATE 38

A.—Seedlings grown in (1) distilled water, (2) 500 parts per million sodium-chlorid solution, (3) 1,000 parts per million sodium-chlorid solution, (4) 2,000 parts per million sodium-chlorid solution, (5) 3,000 parts per million sodium-chlorid solution, and (6) 4,000 parts per million sodium-chlorid solution.

B.—Seedlings grown in sand and watered with (1) distilled water, (2) 500 parts per million sodium-chlorid solution, (3) 1,000 parts per million sodium-chlorid solution, (4) 2,000 parts per million sodium-chlorid solution, (5) 3,000 parts per million sodium-chlorid solution, and (6) 4,000 parts per million sodium-chlorid solution.



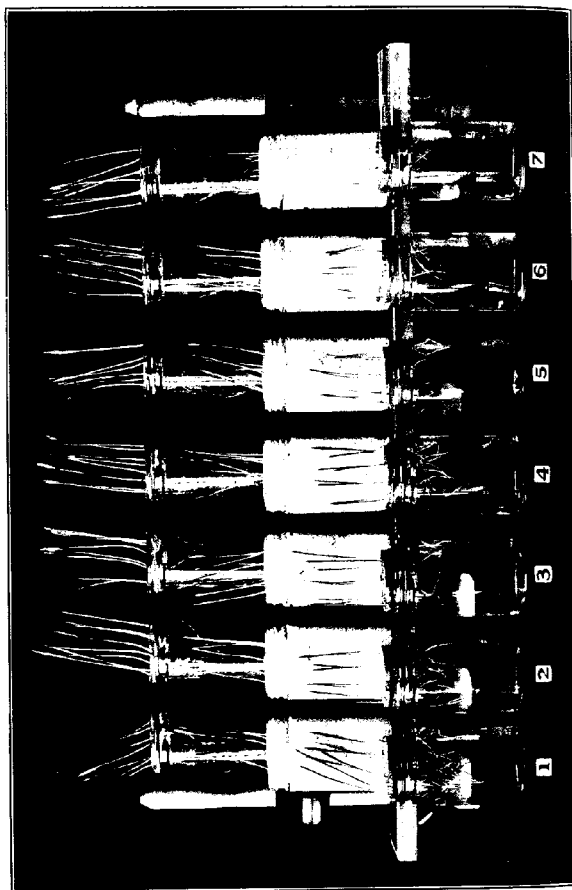


PLATE 39

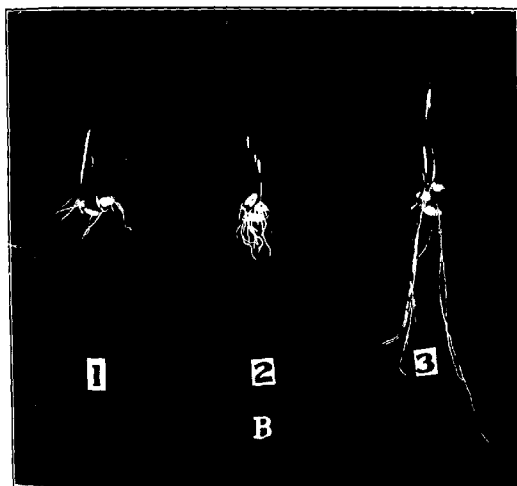
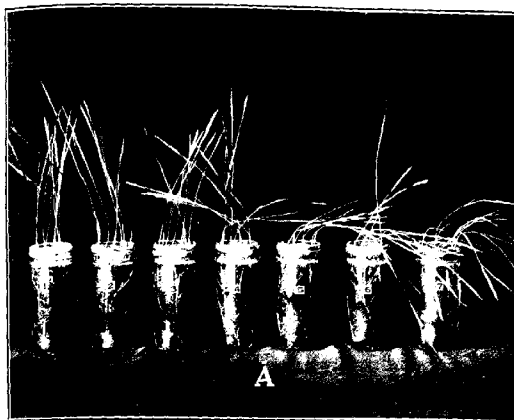
Seedlings grown in water, sand, and clay, showing effects of (1) distilled water, (2) 500 parts per million sodium-chlorid solution, (3) 1,000 parts per million sodium-chlorid solution, (4) 1,500 parts per million sodium-chlorid solution, (5) 2,000 parts per million sodium-chlorid solution, (6) 3,000 parts per million sodium-chlorid solution, and (7) 4,000 parts per million sodium-chlorid solution.



PLATE 40

A.—Seedling, 9 days old, grown in clay watered with (1) distilled water, (2) 500 parts per million sodium-chlorid solution, (3) 1,000 parts per million sodium-chlorid solution, (4) 1,500 parts per million sodium-chlorid solution, (5) 2,000 parts per million sodium-chlorid solution, (6) 3,000 parts per million sodium-chlorid solution, and (7) 4,000 parts per million sodium-chlorid solution.

B.—Seedlings, 3 days old, removed from (1) 4,000 parts per million sodium-chlorid solution, (2) sand watered with 4,000 parts per million sodium-chlorid solution, and (3) clay watered with 4,000 parts per million sodium-chlorid solution.



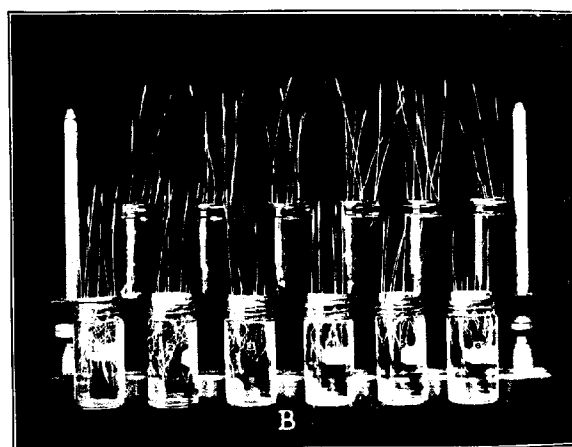
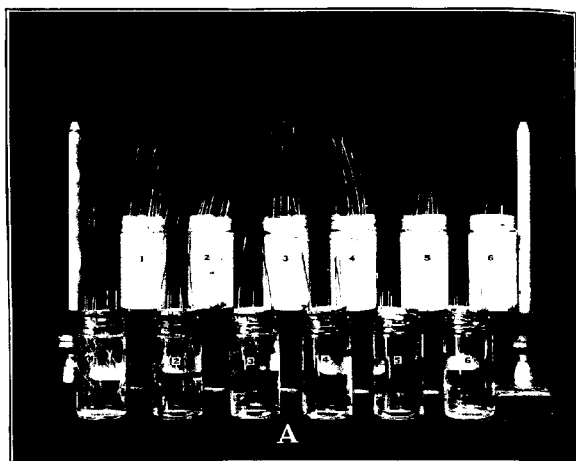


PLATE 41

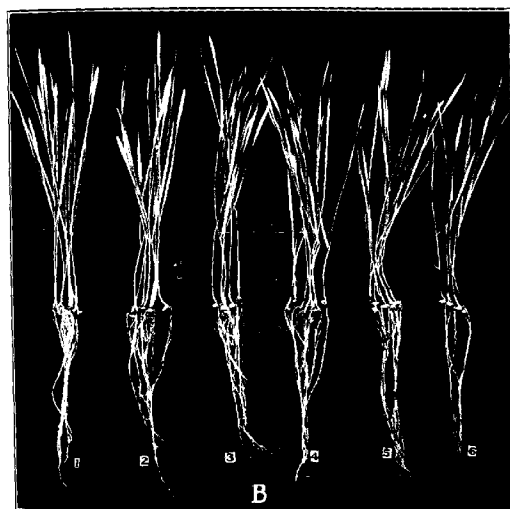
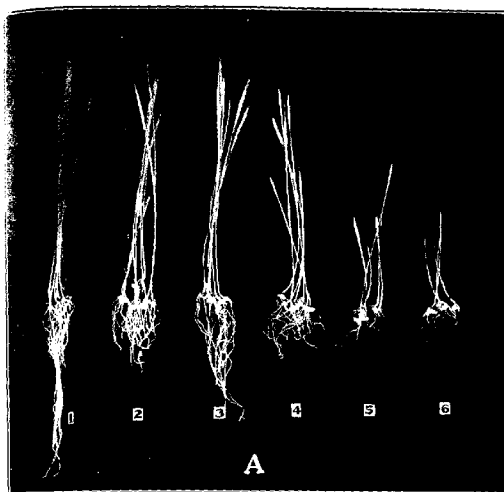
A.—Seedlings grown in sand and in the following solutions filtered through sand: (1) distilled water, (2) 1,000 parts per million of sodium chlorid, (3) 1,500 parts per million of sodium chlorid, (4) 2,000 parts per million of sodium chlorid, (5) 3,000 parts per million of sodium chlorid, and (6) 4,000 parts per million of sodium chlorid.

B.—Seedlings grown in clay and in the following solutions filtered through clay: (1) distilled water, (2) 1,000 parts per million of sodium chlorid, (3) 1,500 parts per million of sodium chlorid, (4) 2,000 parts per million of sodium chlorid, (5) 3,000 parts per million of sodium chlorid, and (6) 4,000 parts per million of sodium chlorid.

PLATE 42

A.—Seedlings, 10 days old, removed from sand watered with (1) distilled water, (2) 1,000 parts per million sodium-chlorid solution, (3) 1,500 parts per million sodium-chlorid solution, (4) 2,000 parts per million sodium-chlorid solution, (5) 3,000 parts per million sodium-chlorid solution, and (6) 4,000 parts per million sodium-chlorid solution.

B.—Seedlings, 10 days old, removed from clay watered with (1) distilled water, (2) 1,000 parts per million sodium-chlorid solution, (3) 1,500 parts per million sodium-chlorid solution, (4) 2,000 parts per million sodium-chlorid solution, (5) 3,000 parts per million sodium-chlorid solution, and (6) 4,000 parts per million sodium-chlorid solution.



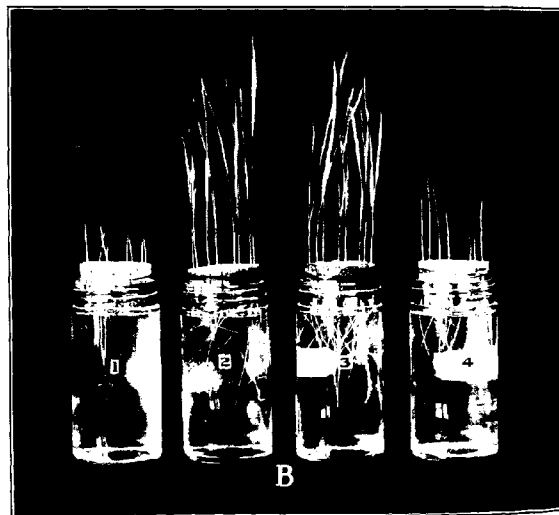
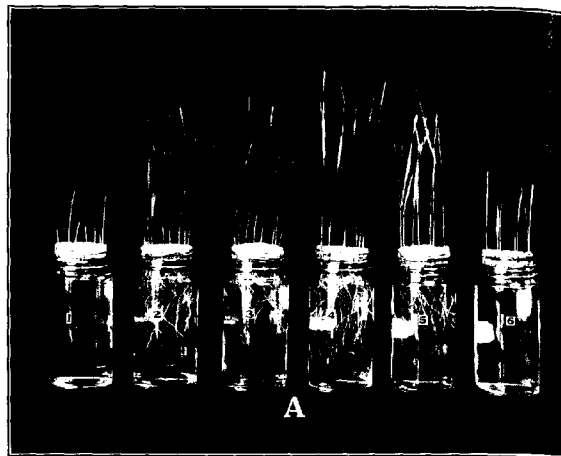


PLATE 43

A.—Seedlings grown in (1) 4,000 parts per million sodium-chlorid solution, (2) 6,000 parts per million sodium-chlorid solution filtered through soil, (3) 8,000 parts per million sodium-chlorid solution filtered through soil, (4) 4,000 parts per million sodium-chlorid solution filtered through soil, (5) 4,000 parts per million sodium-chlorid solution added to distilled water that had previously been filtered through soil, and (6) 4,000 parts per million sodium-chlorid solution + 100 parts per million each of sodium nitrate, potassium chlorid, and sodium phosphate.

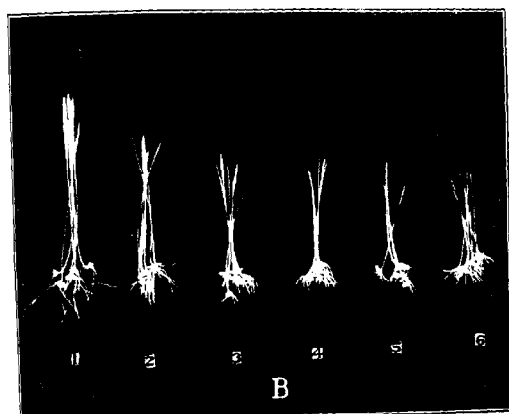
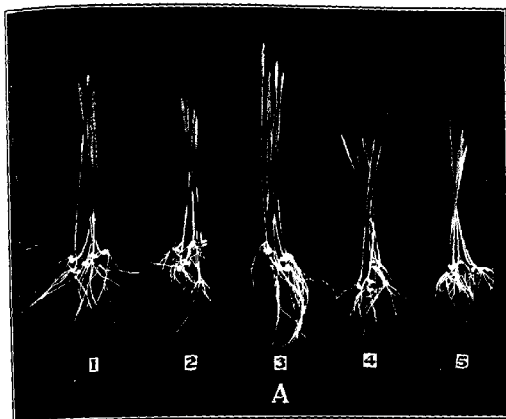
B.—Seedlings grown in (1) 4,000 parts per million sodium-chlorid solution, (2) 4,000 parts per million sodium-chlorid solution + 30 parts per million of calcium sulphate, (3) 4,000 parts per million sodium-chlorid solution + 30 parts per million of calcium oxid, and (4) 4,000 parts per million sodium-chlorid solution + 30 parts per million of magnesium bicarbonate.



PLATE 44

A.—Seedlings grown in (1) distilled water, (2) 4,000 parts per million sodium-chlorid solution + 30 parts per million of calcium sulphate, (3) 4,000 parts per million sodium-chlorid solution + 30 parts per million of calcium oxid, (4) 4,000 parts per million sodium-chlorid solution + 30 parts per million of magnesium sulphate, and (5) 4,000 parts per million sodium-chlorid solution + 30 parts per million of barium chlorid.

B.—Seedlings grown in (1) distilled water, (2) 4,000 parts per million sodium-chlorid solution + 30 parts per million of potassium chlorid, (3) 4,000 parts per million sodium-chlorid solution + 30 parts per million of sodium nitrate, (4) 4,000 parts per million sodium-chlorid solution + 30 parts per million of sodium phosphate, (5) 4,000 parts per million sodium-chlorid solution + 30 parts per million of ferric chlorid, and (6) 4,000 parts per million sodium-chlorid solution + 30 parts per million of potassium alum.



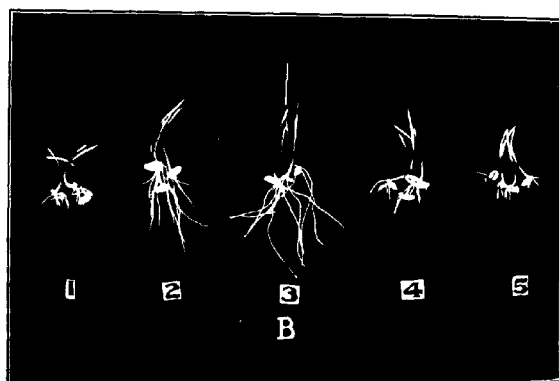
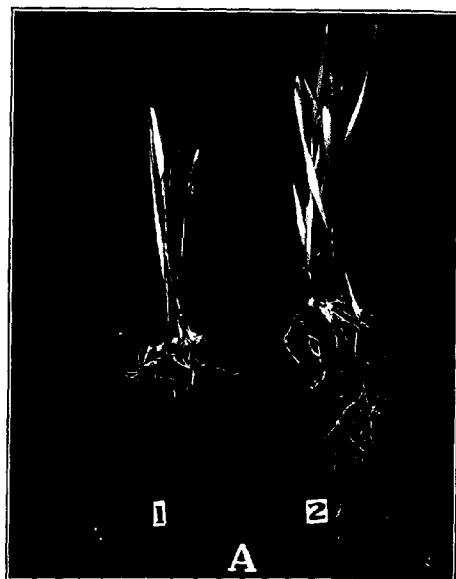


PLATE 45

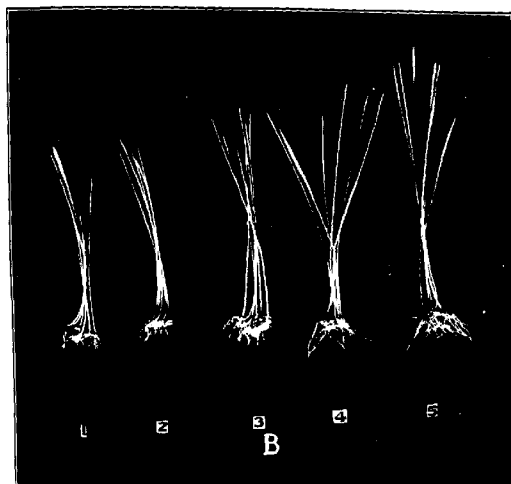
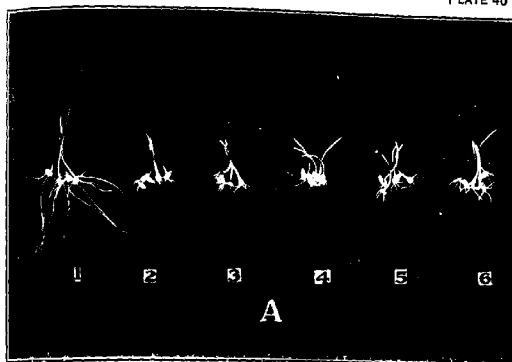
A.—Seedlings grown in sand watered with (1) 4,000 parts per million sodium-chlorid solution and (2) 4,000 parts per million sodium-chlorid solution + 30 parts per million of calcium sulphate.

B.—Seedlings grown in (1) 4,000 parts per million sodium-sulphate solution, (2) 4,000 parts per million sodium-sulphate solution + 30 parts per million of calcium sulphate, (3) 4,000 parts per million sodium-sulphate solution + 30 parts per million of calcium oxid, (4) 4,000 parts per million sodium-sulphate solution + 30 parts per million of magnesium sulphate, and (5) 4,000 parts per million sodium-sulphate solution + 30 parts per million of barium chlorid.

PLATE 46

A.—Seedlings, 3 days old, grown in (1) distilled water, (2) 4,000 parts per million sodium-sulphate solution, (3) 4,000 parts per million sodium-sulphate solution + 30 parts per million of potassium chlorid, (4) 4,000 parts per million sodium-sulphate solution + 30 parts per million of sodium nitrate, (5) 4,000 parts per million sodium-sulphate solution + excess ferric hydrate, and (6) 4,000 parts per million sodium-sulphate solution + aluminum hydrate.

B.—Seedlings, 11 days old, grown in (1) 2,500 parts per million sodium-bicarbonate solution, (2) 2,500 parts per million sodium-bicarbonate solution + 30 parts per million of sodium nitrate, (3) 2,500 parts per million sodium-bicarbonate solution + 30 parts per million of potassium chlorid, (4) 2,500 parts per million sodium-bicarbonate solution + 30 parts per million of magnesium sulphate, and (5) 2,500 parts per million sodium-bicarbonate solution + 30 parts per million of calcium oxid.



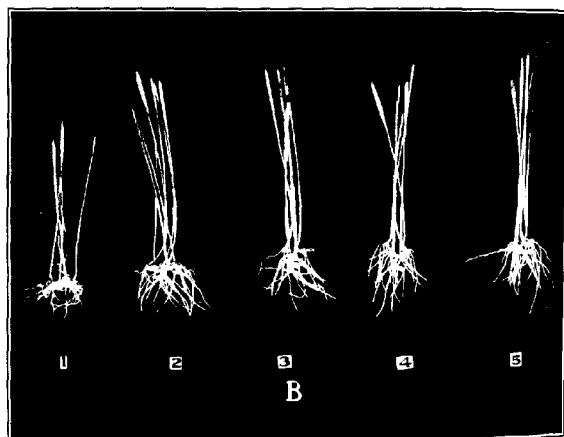
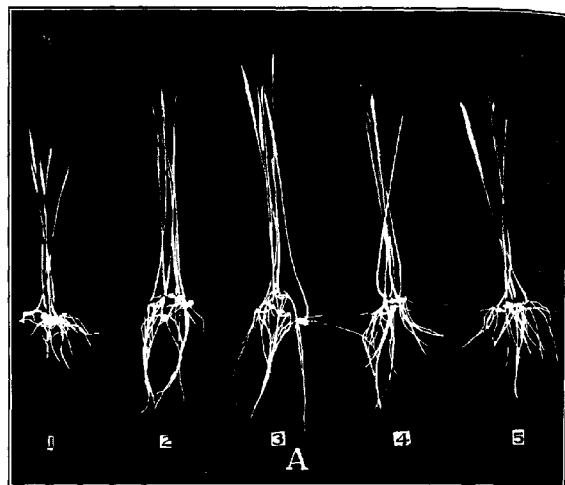


PLATE 47

A.—Seedlings grown in (1) 4,000 parts per million sodium-chlorid solution, (2) distilled water, (3) 4,000 parts per million sodium-chlorid solution + 40 parts per million of calcium oxid, (4) 4,000 parts per million sodium-chlorid solution + 30 parts per million of calcium oxid, and (5) 4,000 parts per million sodium-chlorid solution + 20 parts per million of calcium oxid.

B.—Seedlings grown in (1) 4,000 parts per million sodium-chlorid solution, (2) 4,000 parts per million sodium-chlorid solution + 15 parts per million of calcium oxid, (3) 4,000 parts per million sodium-chlorid solution + 10 parts per million of calcium oxid, (4) 4,000 parts per million sodium-chlorid solution + 2 parts per million of calcium oxid, and (5) 4,000 parts per million sodium-chlorid solution + 1 part per million of calcium oxid.





# RELATION OF MOISTURE IN SOLID SUBSTRATA TO PHYSIOLOGICAL SALT BALANCE FOR PLANTS AND TO THE RELATIVE PLANT-PRODUCING VALUE OF VARIOUS SALT PROPORTIONS

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## INTRODUCTION

Soil moisture is perhaps the most important single factor affecting crop production, and the relation of soil moisture to plant growth in general has been the subject of much investigation. Not so much attention has been given, however, to the relation of moisture in soils or other solid substrata to physiological salt balance or to the plant-producing value of complete fertilizer rations for plants, for the reason perhaps that adequate methods for quantitative studies were not available.

The need of some method by which the effects of nutrient solutions of known composition upon the growth of plants may be studied in the presence of some solid substratum resembling soil, but without so many of the biological and chemical complications always encountered in soil cultures, has practically been realized in the new sand-culture method recently developed by McCall (8).<sup>1</sup> By this method plants may be grown in sand supplied with nutrient solutions of any desired composition, which may be renewed or modified almost as readily as water cultures. This method makes it possible to study quantitatively the influence of various degrees of moisture in solid substrata upon the physiological salt balance, so far as this affects plant growth.

Harris (5) has pointed out that the effect of a fertilizer upon the growth of wheat is largely dependent upon the amount of soil moisture and emphasizes the point that fertilizer experiments, in order to be of any value, must be made under widely varying moisture conditions. It has been shown by Gile (3), McCool (10), Tottingham (15), McCall (9), Ayres (1), and others that the physiological value of any set of salt proportions varies, in general, with the total concentration of the medium; but as yet no general rule has been formulated to express the manner of this variation. It thus appears that physiological salt balance in nutrient solutions is largely dependent upon total concentration.

<sup>1</sup> Reference is made by number (italic) to "Literature cited," p. 377-378.

It has frequently been observed that plants grow less rapidly in a nutrient solution of high total concentration (within certain limits) without toxic effects than they do in a less concentrated solution containing the same constituents in the same relative proportions. It is also a fact of common observation that, regardless of the total concentration of the medium, plants grow less rapidly in a solid substratum, such as soil with low moisture content, than they do in the same medium with an optimum supply of moisture. If, now, a given set of salt proportions, in solution with approximately optimum total concentration for plant growth, should be added to an inert solid medium, such as sand, in such quantities as to produce a very low moisture content, it is obvious that plants would grow less rapidly in such a culture than in a similar culture supplied with the same solution to give an optimum moisture content. Thus, with any given set of salt proportions somewhat similar changes in growth or other forms of plant activity may result (1) from growing the plants in solutions with varying total concentrations of the salts in the given proportions, or (2) from growing the plants in an inert solid substratum, such as sand, supplied with the given set of salt proportions in solutions with constant total concentration so as to produce varying degrees of moisture content. Here any changes in growth with alterations in the degree of moisture would take place independently of the total concentration of the soil (sand) solution.

Since both variations in total concentration of the nutrient medium and alterations in the moisture content of a solid substratum have a somewhat similar influence upon plant activities with respect to retarded or accelerated growth rates, and since it is already well known that the physiological value of any given set of salt proportions is markedly influenced by total concentration, it seemed desirable to determine whether or not the salt balance of nutrient solutions could be similarly influenced or the relative value of a fertilizer treatment be altered by variations in the degree of the moisture content of a solid substratum, such as sand, when the mineral nutrients are diffused as films on the solid particles in the form of solutions of constant total concentrations. The following pages present the result of an experimental study dealing with this question.

#### OUTLINE OF EXPERIMENTAL METHODS

In order to study the effect of differences in the moisture content of a solid substratum upon the physiological salt balance of the nutrient medium, sand to which nutrient solutions were added was here used as the medium in which the plants were grown. The nutrient media added to the sand consisted of the 36 different 3-salt solutions (osmotic concentration value 1.75 atmospheres) comprised in an optimum series previously used with wheat (12) and with buckwheat (13) in studies of physiological salt balance. Three series of these sand cultures were

conducted simultaneously. The three series were alike in every respect except in the quantity of solution which was diffused as a film over the solid sand particles. All the cultures of the same series received the same amount of solution and were kept at approximately the same moisture content; but the cultures of the different series received different amounts of solution, so that the moisture content throughout each series was different from that of the other two series. Thus, corresponding cultures of the three different series received different amounts of the same solution, the differences in their moisture content being regulated by adding more or less solution as the case required.

All the cultures of one series were prepared with a moisture content of approximately 40 per cent of the water-holding capacity of the sand, all the cultures of another series with a moisture content of 60 per cent, and those of the third series with a moisture content of 80 per cent. These values were chosen as the result of preliminary tests which showed that a moisture content of 60 per cent of the water-retaining capacity of the sand used was well within the range for optimum growth, while the first and third values selected were considerably below and above this range.

The substratum used in these cultures consisted of white seashore sand which was thoroughly washed with tap water followed several times with distilled water. This sand had a water-retaining capacity of 25 per cent on the dry-weight basis (average of six tests) determined according to the method of Hilgard (7, p. 209). Thus the moisture contents of the three different series were 10 per cent, 15 per cent, and 20 per cent, respectively, based on the weight of the air-dry sand.

Half-gallon glazed earthenware pots were used as culture vessels. Each pot held 2,500 gm. of dry sand when filled to within several centimeters of the top. To prepare the sand in each pot for the planting of the seedlings, a sufficient amount of nutrient solution was poured into the sand to bring it almost to the point of saturation. Five carefully selected seedlings of spring wheat of the Marquis variety were then transplanted to the sand culture from a germinating net, after which the culture was flooded until free nutrient solution appeared over the surface of the sand to the depth of 1 cm. or more, thus fixing the seedlings in place and at the same time leveling the surface of the sand. This initial application required 750 cc. of solution for each culture. The excess solution was then withdrawn and the sand reduced to the desired moisture content by a method (14) which had previously been described and which is a modification of the method devised by McCall (8) for the renewal of solutions in sand cultures.

The solutions were renewed at 3-day intervals. After each culture had been restored to its original weight by the addition of distilled water, as much as possible of the old solution was withdrawn, and the culture was flooded with 500 cc. of new solution. The culture was then restored

to its original moisture content by withdrawing as much of the new solution as was required to accomplish this. To prevent loss of water by evaporation, all the cultures were sealed at the beginning of the experiment by pouring a thin layer of Briggs and Shantz wax (2) over the surface of the sand around the seedlings.

In all experiments of this kind it is, of course, practically impossible to maintain absolute uniformity in the moisture conditions of the substratum. This ideal condition may not even be very closely approximated because of transpirational water loss, which tends to decrease the moisture content of the cultures and, at the same time to increase the total concentration of the solution. In the present work, however, excessive variation in the moisture content of the cultures and in the total concentrations of the solutions was prevented by the frequent addition of distilled water in quantities sufficient to restore the cultures to their original weights. The cultures were weighed daily, and during the later growth stages whenever the atmospheric conditions were such as to produce high rates of transpiration more frequent weighings were made and the original moisture conditions of the sand cultures were restored by the addition of distilled water. The highest water loss from any culture of the three series during the interval between two successive weighings was not greater than 4 per cent of the original volume of the solution present in the culture; or, on the dry-weight basis, this was not greater than 0.4 per cent for the series with the lowest moisture content, 0.6 per cent for the series with medium moisture content, and 0.8 per cent for the series with highest moisture content. This, of course, represents the extremes in the variations of the moisture conditions. Ordinarily the decrease in the moisture content of the cultures and the consequent increase in the total concentration of the solution during the intervals between two successive weighings were very much less than this. Small variations in the moisture conditions of the substratum, such as were encountered in these sand cultures, could scarcely be expected to have any material influence upon the physiological properties of the solutions as these affect the growth of the plants.

The three series of cultures here considered were conducted simultaneously for a period of 28 days after the seedlings had been transplanted to the sand cultures. The first triple series was conducted from November 30 to December 27, 1917. The second triple series, which was exactly like the first, was carried out between January 18 and February 15, 1918. At the end of the growth period the wax seals were removed from the cultures, the plants harvested in the usual manner, and the dry weights of the tops and roots obtained separately.

# DISCUSSION OF RESULTS

## YIELDS OF TOPS

For convenience in presenting the data, the three series of cultures here considered will be designated series A, series B, and series C, according as the cultures of the series were maintained at a moisture content of 40 per cent, 60 per cent, or 80 per cent of the water-retaining capacity of the sand. Since this triple series of cultures was repeated, two corresponding dry-weight measurements of both tops and roots are available for each culture; and by combining these, the average numerical data given in the tables were obtained.

Table I presents the average dry-weight yields of tops for each of the three moisture contents employed. The first column of each section gives the average absolute yield values in grams, while the second column gives the weights of tops relative to the weights from the first culture ( $R_1C_1$ ) taken as unity. These relative dry-weight values were obtained by averaging the corresponding relative yield values from the two triple series, conducted during different time periods. Each of these relative data is, therefore, the average of two ratios and not the ratio obtained by dividing its corresponding average absolute dry-weight value by the average absolute value of the first culture ( $R_1C_1$ ) in the same series. It thus happens that the relative values given in the table do not always bear exactly the same relation to each other as do the absolute values. The average relative data obtained from the group of cultures which produced the nine highest yields in each series (upper one-fourth) are shown in the table in italics, excepting the highest relative yield value of each series which appears in bold-face type. The culture numbers refer to the positions which the cultures occupy on the triangular diagrams graphically representing the variations in salt proportions and partial osmotic concentrations of the solutions added to the sand cultures.<sup>1</sup>

<sup>1</sup> For descriptions of this triangular diagrammatic scheme see Shive (12), McCall (9), and Hibbard (6). An excellent discussion of the triangle system and its use in problems of plant nutrition has recently been published by Schreiner and Skinner (11).

TABLE I.—Average dry weight of wheat tops grown 28 days in sand cultures supplied with 3-salt solutions, all having an osmotic concentration value of approximately 1.75 atmospheres

Culture No.	Average dry weights of tops (5 plants).					
	Series A, 10 per cent moisture content.		Series B, 15 per cent moisture content.		Series C, 20 per cent moisture content.	
	Absolute.	Relative to R <sub>1</sub> C <sub>1</sub> as unity.	Absolute.	Relative to R <sub>1</sub> C <sub>1</sub> as unity.	Absolute.	Relative to R <sub>1</sub> C <sub>1</sub> as unity.
	Gm.		Gm.		Gm.	
R <sub>1</sub> C <sub>1</sub> .....	0.1734	1.00	0.3211	1.00	0.3437	1.00
C <sub>2</sub> .....	.4347	2.55	.5044	1.57	.3793	1.10
C <sub>3</sub> .....	.3571	2.11	.5238	1.62	.4055	1.20
C <sub>4</sub> .....	.4384	2.54	.5273	1.63	.3754	1.09
C <sub>5</sub> .....	.4904	2.88	.5136	1.59	.4055	1.21
C <sub>6</sub> .....	.4619	2.68	.5289	1.63	.4255	1.23
C <sub>7</sub> .....	.4860	2.80	.4923	1.50	.4103	1.20
C <sub>8</sub> .....	.4851	2.80	.4840	1.50	.4088	1.20
R <sub>2</sub> C <sub>1</sub> .....	.2887	1.97	.3647	1.15	.3680	1.12
C <sub>2</sub> .....	.4739	2.77	.5106	1.60	.4350	1.32
C <sub>3</sub> .....	.5352	3.06	.4858	1.49	.3893	1.18
C <sub>4</sub> .....	.4793	2.74	.4750	1.48	.3780	1.14
C <sub>5</sub> .....	.5772	3.31	.5285	1.63	.4161	1.23
C <sub>6</sub> .....	.5164	3.05	.4654	1.44	.3630	1.20
C <sub>7</sub> .....	.4622	2.66	.4300	1.36	.3556	1.03
R <sub>3</sub> C <sub>1</sub> .....	.3330	1.97	.4091	1.26	.4069	1.22
C <sub>2</sub> .....	.4496	2.60	.5363	1.63	.3993	1.13
C <sub>3</sub> .....	.5793	3.36	.5392	1.66	.4320	1.28
C <sub>4</sub> .....	.4919	2.82	.4354	1.35	.4414	1.32
C <sub>5</sub> .....	.5075	3.41	.4782	1.49	.3656	1.08
C <sub>6</sub> .....	.4371	2.45	.4192	1.31	.3336	1.02
R <sub>4</sub> C <sub>1</sub> .....	.3475	2.05	.4220	1.32	.4303	1.30
C <sub>2</sub> .....	.5256	3.06	.5623	1.76	.4632	1.37
C <sub>3</sub> .....	.4879	2.82	.6756	2.07	.4786	1.43
C <sub>4</sub> .....	.5615	3.22	.5931	1.83	.4236	1.27
C <sub>5</sub> .....	.5275	3.04	.6353	2.00	.3804	1.15
R <sub>5</sub> C <sub>1</sub> .....	.4095	2.37	.4687	1.48	.4618	1.38
C <sub>2</sub> .....	.6390	3.67	.8354	2.68	.6001	1.75
C <sub>3</sub> .....	.6240	3.56	.7933	2.17	.5874	1.76
C <sub>4</sub> .....	.5856	3.37	.6852	2.13	.4778	1.43
R <sub>6</sub> C <sub>1</sub> .....	.4705	2.78	.5391	1.67	.4915	1.47
C <sub>2</sub> .....	.5666	3.29	.6645	2.03	.4993	1.44
C <sub>3</sub> .....	.5402	3.13	.6072	1.88	.5475	1.62
R <sub>7</sub> C <sub>1</sub> .....	.4510	2.68	.6015	1.87	.5415	1.59
C <sub>2</sub> .....	.5114	2.95	.6398	1.99	.5502	1.62
R <sub>8</sub> C <sub>1</sub> .....	.4302	2.53	.5727	1.79	.5213	1.55

Table II presents the average data of root yields corresponding to those of top yields in Table I.

TABLE II.—Average dry weight of wheat roots grown 28 days in sand cultures supplied with 3-salt solutions, all having an osmotic concentration value of approximately 1.75 atmospheres

Culture No.	Average dry weights of tops (5 plants).					
	Series A, 10 per cent moisture content.		Series B, 15 per cent moisture content.		Series C, 20 per cent moisture content.	
	Absolute.	Relative to R <sub>1</sub> C <sub>1</sub> as unity.	Absolute.	Relative to R <sub>1</sub> C <sub>1</sub> as unity.	Absolute.	Relative to R <sub>1</sub> C <sub>1</sub> as unity.
	Gm.		Gm.		Gm.	
R <sub>1</sub> C <sub>1</sub> .....	0.0903	1.00	0.1750	1.00	0.1546	1.00
C <sub>2</sub> .....	.2013	2.27	.2475	1.40	.1895	1.22
C <sub>3</sub> .....	.1829	2.10	.2645	1.50	.1972	1.26
C <sub>4</sub> .....	.2263	2.54	.2893	1.64	.1860	1.17
C <sub>5</sub> .....	.2332	2.63	.2506	1.41	.2004	1.29
C <sub>6</sub> .....	.2050	2.33	.2698	1.53	.2031	1.28
C <sub>7</sub> .....	.2129	2.42	.2593	1.44	.2034	1.28
C <sub>8</sub> .....	.2153	2.42	.2414	1.36	.2235	1.42
R <sub>2</sub> C <sub>1</sub> .....	.1475	1.69	.1964	1.12	.1672	1.08
C <sub>2</sub> .....	.2322	2.57	.2528	1.42	.2013	1.30
C <sub>3</sub> .....	.2247	2.53	.2600	1.45	.1815	1.19
C <sub>4</sub> .....	.2141	2.46	.2279	1.29	.1777	1.17
C <sub>5</sub> .....	.2506	2.69	.3173	1.78	.2008	1.33
C <sub>6</sub> .....	.2481	2.87	.3099	1.74	.2259	1.46
C <sub>7</sub> .....	.2360	2.68	.2920	1.68	.2072	1.31
R <sub>3</sub> C <sub>1</sub> .....	.1622	1.82	.2196	1.24	.1855	1.21
C <sub>2</sub> .....	.1965	2.17	.2378	1.33	.1757	1.12
C <sub>3</sub> .....	.2225	2.45	.2823	1.58	.1963	1.26
C <sub>4</sub> .....	.2174	2.39	.2597	1.47	.2221	1.42
C <sub>5</sub> .....	.2245	2.60	.3165	1.81	.2166	1.37
C <sub>6</sub> .....	.2363	2.54	.3254	1.87	.1885	1.18
R <sub>4</sub> C <sub>1</sub> .....	.1708	1.89	.2267	1.29	.1603	1.10
C <sub>2</sub> .....	.2159	2.39	.2879	1.62	.2021	1.31
C <sub>3</sub> .....	.2285	2.51	.3023	1.69	.1982	1.26
C <sub>4</sub> .....	.2619	2.96	.3230	1.84	.2378	1.50
C <sub>5</sub> .....	.2824	3.17	.3998	2.26	.2131	1.38
R <sub>5</sub> C <sub>1</sub> .....	.1813	1.99	.2077	1.19	.1975	1.28
C <sub>2</sub> .....	.2542	2.77	.3439	1.93	.2400	1.53
C <sub>3</sub> .....	.2545	2.76	.3231	1.82	.2609	1.68
C <sub>4</sub> .....	.2663	2.94	.3603	2.06	.2860	1.84
R <sub>6</sub> C <sub>1</sub> .....	.2002	2.24	.2455	1.39	.1900	1.24
C <sub>2</sub> .....	.2365	2.62	.3012	1.69	.2443	1.53
C <sub>3</sub> .....	.2456	2.69	.3370	1.94	.2431	1.59
R <sub>7</sub> C <sub>1</sub> .....	.1709	2.04	.2685	1.52	.2599	1.67
C <sub>2</sub> .....	.2453	2.75	.2459	1.42	.2469	1.58
R <sub>8</sub> C <sub>1</sub> .....	.1904	2.13	.2709	1.58	.2392	1.52

The relative yield values of tops and of roots, taken directly from the proper columns of averages in the tables, were plotted on the triangular diagrams, as was done in earlier publications and by other writers. Since, however, the medium and low yields are of little interest in this connection, only that group of nine cultures in each series which produced the highest yields (upper one-fourth) will be considered in this discussion. On the diagrams of figure 1 areas are outlined to show the distribution of the 9 highest yield values of tops for each of the three different series of 36 cultures. The region or regions in each diagram including these nine high-yielding cultures are indicated by shaded areas. The highest-yielding culture in each series is marked by a circle.



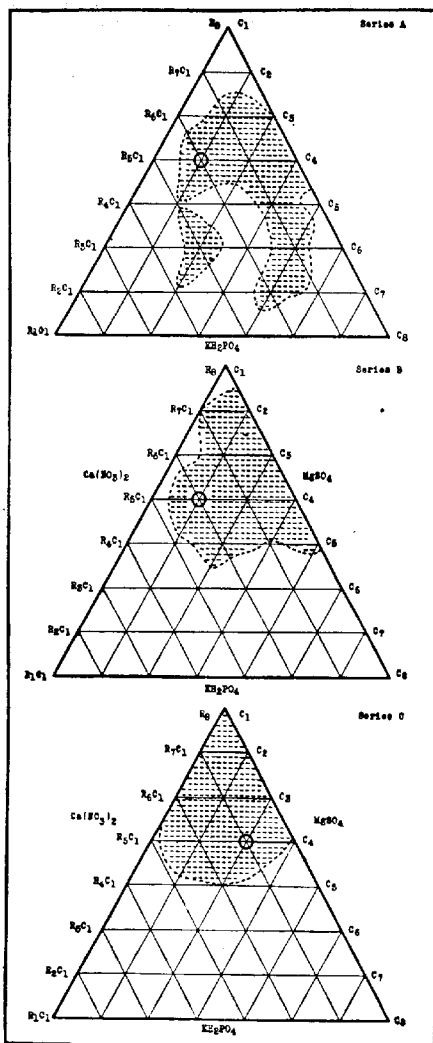


FIG. 1.—Diagrams showing the position of the cultures producing the nine highest yields of wheat 1009 in each series. The cultures giving maximum yields are marked by circles.

From a comparison of the three diagrams of figure 1 it is at once apparent that there is a marked degree of similarity between the diagrams with respect to the locations of the areas of good growth. Out of a group of nine cultures in each series giving high yield values, five are corresponding cultures of the three series and are included in the areas marking good growth in each of the three diagrams. These five cultures are R<sub>4</sub>C<sub>4</sub>, R<sub>5</sub>C<sub>2</sub>, R<sub>5</sub>C<sub>3</sub>, R<sub>6</sub>C<sub>2</sub>, and R<sub>6</sub>C<sub>3</sub>. The two cultures R<sub>7</sub>C<sub>1</sub> and R<sub>7</sub>C<sub>2</sub> are also included in the group of high-yielding cultures in both series B and C, as is indicated on the diagrams representing these two series.

The highest average dry-weight yields obtained from series A and from series B were produced by corresponding cultures (R<sub>5</sub>C<sub>2</sub>) of the two series. The highest average relative yield obtained from series C was that produced by culture R<sub>5</sub>C<sub>3</sub>. It is to be noted, however, that during the two experimental periods of this series cultures R<sub>5</sub>C<sub>2</sub> and R<sub>5</sub>C<sub>3</sub> produced corresponding dry-weight yields which were nearly equal in value, so that the average absolute yield from the former was somewhat higher than that from the latter, while the average relative yield from the latter was slightly higher than that of the former.

A careful comparison of the diagrams of the three yields with reference to the location of the high-producing cultures brings out the fact that these areas approach more closely to the apex of the triangle and recede correspondingly from the base as the moisture content of the cultures in the different series is increased. Thus, in series A, which was maintained at a moisture content of 40 per cent of the water-retaining capacity of the sand, the areas of high yields lie mainly in a central region of the triangle between the second row of cultures from the base and the third row from the apex. In series B, which had a 60 per cent moisture content, the area of high yields is centrally located, mainly between rows 4 and 7. While this area has receded considerably from the base of the triangle toward the apex as compared with the areas of high yields in series A, it does not extend entirely to the apex and just touches the left margin at culture R<sub>7</sub>C<sub>1</sub>. In series C, which had an 80 per cent moisture content, the area of high yields has receded still farther from the base of the triangle and extends entirely to the apex, bordering on both right and left margins.

From the foregoing facts it is at once clear that the differences in the degrees of moisture employed in the cultures of these 3 series had no apparent influence upon the physiological salt balance of the culture producing maximum yields of tops, since the salt proportions and the total concentrations of the soil (sand) solutions of the highest-yielding cultures of these series were approximately the same, and since the cultures occupied the same relative positions in their respective series with reference to the yields produced, as is indicated in Table I and on the diagrams of figure 1. The fact that out of each of the 3 groups

of 9 high-yielding cultures 5 are corresponding cultures of the 3 series, as is indicated on the diagrams representing high yields of tops, still further points to the conclusion that good salt balance of nutrient solutions for wheat tops is not markedly disturbed when the solutions are diffused as films on the solid particles of an inert substratum in such a manner as to produce even large differences in the degrees of moisture. It is to be noted, however, that there is a slight but general shifting of the physiological salt balance for the group of 9 high-yielding cultures, as a whole, with each increase in moisture content, from a position in the series characterized by lower partial concentrations of potassium phosphate ( $\text{KH}_2\text{PO}_4$ ) to one of higher partial concentrations of this salt and correspondingly lower ones of calcium nitrate ( $\text{Ca}(\text{NO}_3)_2$ ) and magnesium sulphate ( $\text{MgSO}_4$ ). Thus with the 36 different sets of salt proportions here employed in sand cultures, and with approximately constant total concentrations of the nutrient media, the best physiological salt balance with the lowest moisture content used was also the best with the medium and with the highest moisture content of the solid substratum.

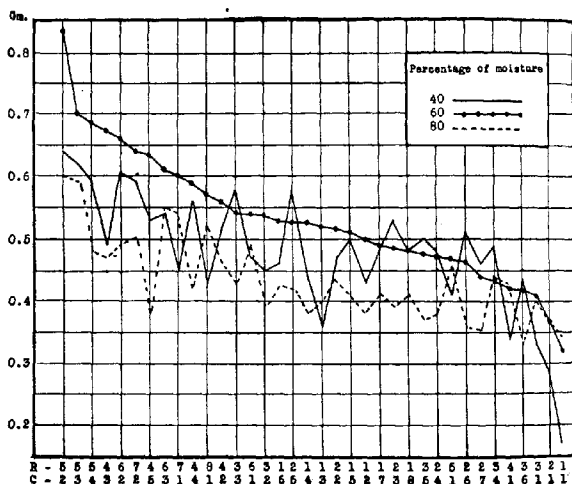


FIG. 2.—Average absolute yields of wheat tops for low, medium, and high moisture content of sand cultures.

The foregoing consideration of relative yields was not intended to show the effect of differences in degrees of moisture content upon the actual growth rates as indicated by the absolute dry-weight yields. To bring out the relation in question, the average absolute dry-weight yield values

of each series, taken from the columns of Table I, were arranged in the descending order of the values obtained from series B, employing a medium moisture content. These values were then plotted to form the three graphs shown in figure 2, representing the three different series of absolute dry-weight values. The continuous line marked by large dots and sloping somewhat uniformly downward to the right represents series B, with medium moisture content, while the broken line and the plain continuous line represent the yields obtained from the series with the highest and the lowest moisture contents, respectively.

Inspection of figure 2 shows that each of the three graphs has a decided tendency to slope downward to the right, thus indicating, in a general way, changes in the growth rates with variations in the salt proportions. In this respect the three series show a general agreement, which was brought out also by the triangular diagrams. The graph representing the series with medium moisture content lies above the other two graphs throughout most of its length. In the upper third of its course this graph shows the average absolute yields to be much higher than the corresponding yields from the other two series. However, that portion of the graph representing medium and low yields is intersected at various points by the other graphs. Nine yields from series A, with lowest moisture content, and three yields from series C, with highest moisture content, are thus shown to have higher values than the corresponding yields from series B.

The graphs of series A and series C are quite irregular and show little tendency toward parallelism. Practically the only tendency toward any agreement, in this respect, between these 2 series is shown for the first 5 cultures, which appear to rise and fall simultaneously. It will be observed, however, that more than two-thirds of the yields from series A are higher than the corresponding yields from series C. The highest yield from series C is lower than the highest from series A, and both are considerably lower than the highest yield from series B. In fact, the first 12 cultures of series B, as these cultures are arranged for the graphs of figure 2, show yield values which are considerably above the corresponding yield values of the other 2 series. This is somewhat striking in connection with the fact that series A and series C represent the extremes in the moisture content employed.

Perhaps the most important point brought out by the foregoing consideration of the absolute dry-weight values is the fact that the differences in the growth rates brought about by the variations in the moisture content, as indicated by the differences in the yield values of the corresponding cultures of the three series, are nearly as marked as are the differences in the rates of growth resulting from variations in the salt proportions throughout each series. This emphasizes the importance of a constant moisture supply in all pot-culture work of this kind, where the influence of relative salt proportions or of fertilizer treatments is

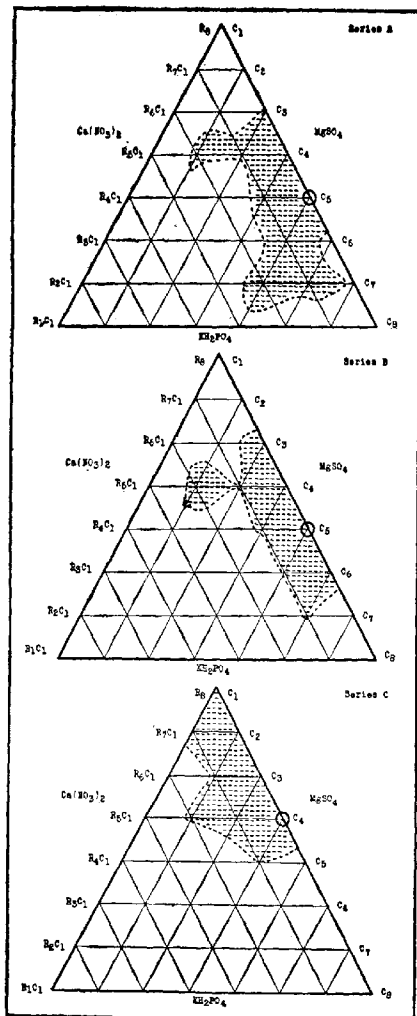


FIG. 3.—Diagrams showing the position of the cultures producing the nine highest yields of wheat roots in each series. The cultures giving maximum yields are marked by circles.

the factor under investigation. It appears that correct interpretations of the influence upon plant growth of such variables as the relative salt proportions here considered are not possible unless the moisture supply of the substratum in which the plants are rooted is maintained within very narrow variation limits.

#### YIELDS OF ROOTS

The position of the group of nine cultures which produced the highest average dry-weight yields of roots in each series is indicated by the shaded areas on the triangular diagrams of figure 3. This diagrammatic arrangement is in every respect similar to that of figure 1, which shows the distribution of high top yields. A comparison of the triangular diagrams of figure 3 shows the agreements between areas marking high root yields to be even more pronounced than are those between the corresponding areas representing the high yields of tops. Out of a total of nine cultures in each series which produced high yields of roots, five are corresponding cultures of the three series, as is indicated on the diagrams. These cultures are R<sub>4</sub>C<sub>4</sub>, R<sub>5</sub>C<sub>2</sub>, R<sub>5</sub>C<sub>3</sub>, R<sub>5</sub>C<sub>4</sub>, and R<sub>6</sub>C<sub>3</sub>. It will be observed also that eight corresponding cultures are represented in the areas of high root yields in both series A and series B. The highest yield of roots in each of these two series was obtained from culture R<sub>4</sub>C<sub>5</sub>, while in series C the highest yield was produced by culture R<sub>5</sub>C<sub>4</sub>.

Series A and series B, with low and medium moisture contents, respectively, show almost absolute agreement with respect to the location of the areas of high root yields. These areas occupy central positions on the right margins of the diagrams, while the corresponding area on the diagram of series C, with the highest moisture content, is shown to occupy a region on the right and left margins of the triangle, farther from the base and extending to the apex. It is thus evident that there is scarcely any shifting of the physiological balance of salt proportions for the group of nine cultures giving the highest yields of roots in passing from the low to the medium moisture content, but with the highest moisture content the physiological salt balance characterizing the nine high-yielding cultures shows the same tendency to migrate toward the apex of the triangle as did that of the nine cultures which produced high yields of tops in the same series. But this shifting of the physiological salt balance characterizing good yields of roots, with a change from the low to the high moisture content, is scarcely more pronounced than is that of the salt balance characterizing good top yields, since the same number of high root yields as of high top yields in each group of nine occurred with corresponding cultures in the three series.

The average absolute dry-weight yields of wheat roots for each of the three different degrees of moisture employed in the sand cultures are shown graphically in figure 4, in the same manner as were the corresponding yields of tops in the graphs of figure 2.

The dry-weight yields of the series employing a medium moisture content (series B) were arranged in the order of their values, beginning with the highest. These are represented in the upper graph of figure 4. The yield values of series A and series C were plotted in the same order,

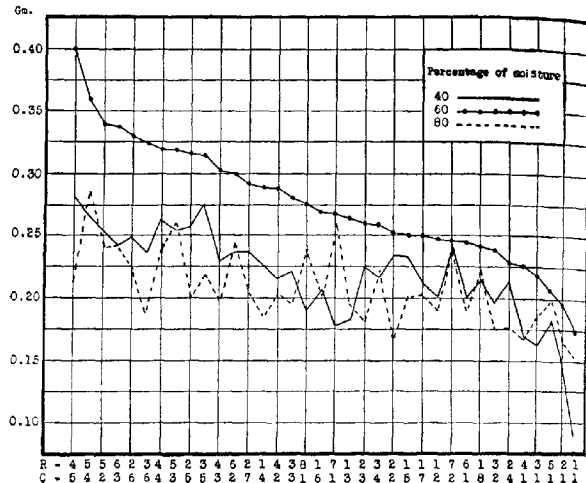


FIG. 4.—Average absolute yields of wheat roots for low, medium, and high moisture content of sand cultures.

and these are represented by the graphs indicated by the continuous and the broken line, respectively. As in the graphs representing the yields of tops, there is a decided tendency for each of these graphs to slope downward to the right, thus indicating the difference in the yield values brought about mainly by the variations in the salt proportions. But this tendency is much more marked in series B than it is in either of the other two series. The medium moisture content (series B) shows the highest absolute dry weights of roots throughout the entire series. It is to be noted also that the highest yields in the two series with the extremes in the moisture content employed are nearly equal in value and very much lower than the highest yield produced with medium moisture content.

The differences between the graphs representing the root yields from the two series of cultures with the lowest and the highest moisture content are not pronounced; these two series resemble each other with re-

spect to the actual root yields produced more than either one resembles the series with medium moisture content. This particular relation between these two series was also apparent but less marked in the graphs representing the dry-weight yields of tops; and, as previously remarked, it is of especial interest in connection with the fact that these two series represent the extremes of moisture content. The graphs of these two series intersect at various points, but there is no marked tendency for the yield values, as a whole, to be either higher or lower with one series than with the other. From an *a priori* consideration of the problem, however, this is not what might be expected. It appears that the growth rates of both tops and roots are considerably retarded by low moisture content. This is unquestionably the result of greater resistance to water absorption by the plant roots, resulting in an internal water supply deficient for optimum growth.

It might be expected that with sand cultures such as were here employed, with approximately constant total concentrations of the nutrient media, a progressive increase in the moisture content up to the point of saturation as a limit to the moisture variation would correspondingly accelerate the growth rates of the plants because of a decreased resistance to water absorption. This, however, does not occur, as the graphs of figures 2 and 4 clearly show. It appears that the growth rates are accelerated by an increase in the moisture content of the sand cultures up to a certain optimum, after which with further increase in the moisture content there is a marked retardation in the rates of growth. This is to be attributed to other factors unfavorable to growth, which are introduced with increased moisture content above the optimum. Whatever the nature of these factors may be, it is clear, as has been brought out, that a sand culture supplied with a well-balanced nutrient solution to give an optimum moisture content is much superior in plant-producing power to a similar sand culture supplied with the same solutions to produce a moisture content closely approaching the point of saturation. In this connection Hall, Brenchley, and Underwood (4) have pointed out that growth in nutrient solutions diffused as films over sand particles is much superior to that in water cultures with the same solutions, but the growth in water cultures is similarly increased when a continuous air current is passed through the solutions. They ascribe enormous advantages to the plants from continuous aeration and attribute to this factor alone the superiority of growth in solid substrata over that in the ordinary water cultures in which aeration is not continuous.

The fact that the average dry-weight yields of both tops and roots from the best nine cultures of the series employing a medium moisture content are always considerably higher than are the corresponding yields from the series with the lowest and highest moisture contents here used clearly shows that well-balanced solutions with optimum total concentrations for plant growth are not alone sufficient to produce the best



yields of which these solutions are capable when they are diffused as films on the particles of a solid substratum, such as sand, and in quantities in excess of the requirements of the plants. An optimum degree of moisture under such conditions is essential to impart to the soil (sand) solution its maximum physiological value. It appears that the actual plant-producing power of any given set of salt proportions or of any fertilizer treatment is largely determined by the moisture conditions of the substratum.

#### INFLUENCE OF MOISTURE CONTENT OF SAND CULTURES UPON TRANSPIRATION AND WATER REQUIREMENT

Throughout the growth period the transpirational water loss from each culture during the intervals between successive weighings was recorded. The total water loss from each culture was then determined by summing the partial losses thus recorded for the entire growth period. The ratio

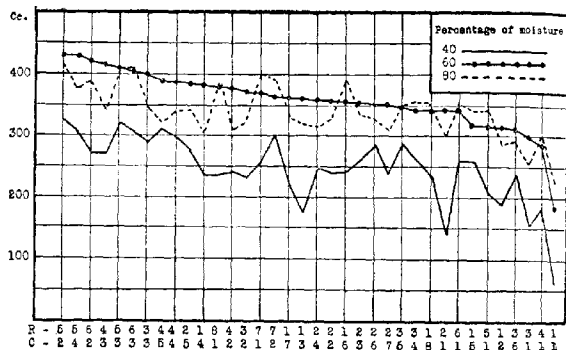


FIG. 5.—Amounts of water lost by transpiration from wheat plants grown in sand cultures with low, medium, and high moisture content.

between the amount of water lost by transpiration during the entire growth period and the dry weight of tops and of roots produced during the same period were calculated for each culture. These ratios, which are quantitative measures (expressed in cubic centimeters) of the water lost by transpiration during the production of a single gram of dry tops or dry roots, as well as the total water loss from each culture of the three series, are presented in Table III. Each value in the table represents the average of corresponding data obtained from duplicate pairs of cultures of the repeated series.

TABLE III.—Transpirational water loss and amounts of water required for the production of each gram of tops and of roots (water requirement)

Culture No.	Transpiration.			Water requirement.								
	Series A.	Series B.	Series C.	Tops.			Roots.			Series A.	Series B.	Series C.
				Series A.	Series B.	Series C.	Series A.	Series B.	Series C.			
R <sub>1</sub> Cr.	C <sub>6</sub>	C <sub>6</sub>	C <sub>6</sub>	C <sub>6</sub> <sup>a</sup>	C <sub>6</sub> <sup>a</sup>	C <sub>6</sub> <sup>a</sup>	C <sub>6</sub> <sup>a</sup>	C <sub>6</sub> <sup>a</sup>	C <sub>6</sub> <sup>a</sup>	C <sub>6</sub> <sup>a</sup>	C <sub>6</sub> <sup>a</sup>	C <sub>6</sub> <sup>a</sup>
C <sub>2</sub>	66	184	227	382	573	661	733	1,051	1,403			
C <sub>3</sub>	102	318	280	442	631	762	955	1,288	1,530			
C <sub>4</sub>	178	361	321	498	688	791	977	1,302	1,630			
C <sub>5</sub>	234	382	305	534	735	814	1,035	1,322	1,640			
C <sub>6</sub>	259	319	341	519	621	840	1,111	1,276	1,795			
C <sub>7</sub>	243	326	390	526	673	617	1,180	1,318	1,920			
C <sub>8</sub>	228	362	333	468	736	812	1,071	1,447	1,640			
R <sub>2</sub> Cr.	238	344	353	491	711	862	1,107	1,438	1,575			
C <sub>2</sub>	143	244	300	395	668	815	967	1,246	1,705			
C <sub>3</sub>	242	357	328	510	688	754	1,042	1,411	1,632			
C <sub>4</sub>	258	355	334	482	730	858	1,146	1,365	1,835			
C <sub>5</sub>	240	358	316	520	753	836	1,104	1,571	1,776			
C <sub>6</sub>	274	386	341	475	731	820	1,067	1,217	1,680			
C <sub>7</sub>	286	354	330	554	761	670	1,153	1,213	1,460			
C <sub>8</sub>	241	352	312	521	802	877	1,022	1,205	1,508			
R <sub>3</sub> Cr.	157	302	308	471	738	756	968	1,371	1,655			
C <sub>2</sub>	233	372	320	518	694	802	1,183	1,562	1,818			
C <sub>3</sub>	286	400	354	494	742	820	1,272	1,418	1,706			
C <sub>4</sub>	260	345	356	520	793	807	1,108	1,326	1,602			
C <sub>5</sub>	289	349	349	570	730	954	1,051	1,100	1,586			
C <sub>6</sub>	244	316	295	538	754	884	1,033	973	1,563			
R <sub>4</sub> Cr.	185	288	307	532	682	713	1,083	1,270	1,815			
C <sub>2</sub>	242	378	353	460	673	762	1,120	1,312	1,750			
C <sub>3</sub>	270	415	344	552	614	718	1,180	1,375	1,738			
C <sub>4</sub>	299	390	336	532	658	792	1,141	1,204	1,412			
C <sub>5</sub>	311	394	321	500	620	845	1,102	985	1,508			
R <sub>5</sub> Cr.	213	318	341	522	678	739	1,207	1,530	1,721			
C <sub>2</sub>	324	429	417	507	514	605	1,276	1,247	1,730			
C <sub>3</sub>	320	410	409	512	573	607	1,256	1,274	1,566			
C <sub>4</sub>	308	428	376	526	625	787	1,158	1,188	1,315			
R <sub>6</sub> Cr.	262	344	356	556	638	724	1,310	1,398	1,872			
C <sub>2</sub>	270	416	392	476	627	800	1,138	1,383	1,607			
C <sub>3</sub>	305	406	406	565	669	741	1,250	1,206	1,670			
R <sub>7</sub> Cr.	257	371	404	570	617	746	1,452	1,378	1,554			
C <sub>2</sub>	290	363	390	584	638	710	1,221	1,475	1,580			
R <sub>8</sub> Cr.	236	380	384	550	604	737	1,243	1,372	1,668			

<sup>a</sup> Quantity of water computed as required for production of 1 gm. of dry tops or roots.

In order to bring out the relation between the moisture content of the sand cultures and the amounts of water lost by transpiration the average actual amounts of water lost, as given in the table, were plotted to form the graphs of figure 5. These graphs, as well as those in figures 6 and 7 which show the water requirement, were prepared in the same manner as were those representing the yields of tops and of roots (fig. 2, 4). The actual average losses from the cultures of series B, which had a medium moisture content, arranged in the descending order of their magnitudes are represented by the full line marked by large dots and sloping uniformly downward to the right. The average losses from the cultures of the other two series were then arranged in the same order and were plotted on the same scale.

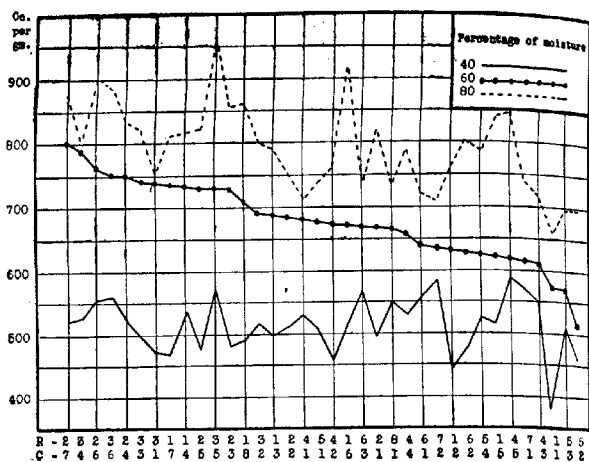


FIG. 6.—Water requirement (in cubic centimeters per gram) of wheat tops grown in sand cultures with low, medium, and high moisture content.

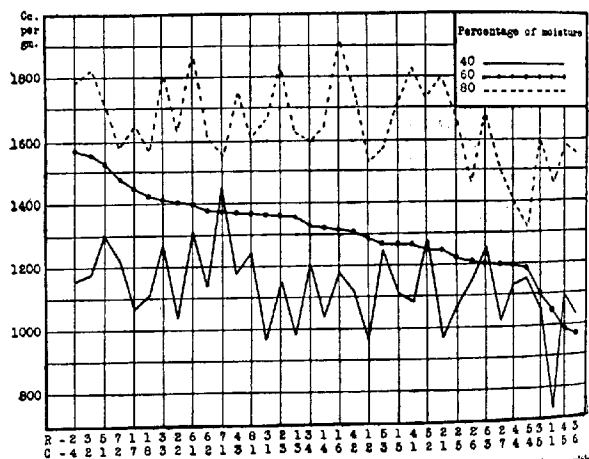


FIG. 7.—Water requirement (in cubic centimeters per gram) of wheat roots grown in sand cultures with low, medium, and high moisture content.

It will be observed that the cultures of the series having the lowest moisture content throughout the experiment exhibit a much lower water loss than do the corresponding cultures of the other two series. There is no such clearly defined relation, however, between the actual amounts of water lost and the highest and medium degrees of moisture employed with the cultures of series B and series C, respectively. There appears to be no general tendency for the transpiration to be either higher or lower with the medium moisture content than with the highest.

A comparison of the transpiration graphs with those representing the yields of tops and of roots (fig. 2, 4), brings out several interesting relations. Low moisture content of the sand cultures denotes low yields of tops and of roots and low transpiration, while a high moisture content corresponds to low yields of tops and of roots but high transpiration. Thus, while low soil (sand) moisture retards both the rate of growth and transpiration, excessive moisture of the substratum, on the other hand, markedly retards the growth rates but does not correspondingly retard transpiration. The medium moisture content, 60 per cent of the water-retaining capacity of the substratum, gave the highest yields of both tops and roots and the highest transpiration.

The water requirement of tops and of roots is graphically shown in figures 6 and 7, respectively, in the same manner as are the yields of tops and of roots and the actual amounts of water lost by transpiration (fig. 2, 4, 5).

Inspection of the graphs of water requirement of tops and of roots shows at once that the values of the water requirement ratios increase with each increase in the moisture content of the substratum. Thus, from the positions of the graphs it is clear that the values of the water requirement ratios are determined by the moisture conditions of the cultures. High, medium, and low moisture content of the substratum is correlated with high, medium, and low water requirement ratios, respectively, of both tops and roots. This relation is perfectly definite for the wheat tops of these tests and, in the main, also for the roots, although several cultures with the lowest moisture content (series A) show water requirement ratios which are slightly higher than are those of the corresponding cultures with medium moisture content (series B), as is indicated on the graphs of figure 7.

As has previously been pointed out, both the highest and the lowest moisture content here employed retarded the growth rates, the former through some harmful influence (perhaps insufficient aeration) related to excessive moisture, the latter undoubtedly through the resistance offered to water absorption by the plant roots, which resulted in an insufficient internal water supply necessary for good growth. The transpiration graphs show, however, that with the highest moisture content (series C) the transpiration rates were not correspondingly retarded, so that under these conditions a comparatively large amount

of water was required to produce a single gram of dry plant material, and the water requirement ratios are high for this series. With the lowest moisture content, on the other hand, the transpiration rates were retarded proportionately more than were the growth rates. Thus for each gram of dry plant substance produced a relatively small quantity of water was required, and the water-requirement ratios for this series are low. A further comparison of the graphs representing water requirement of tops and of roots with the corresponding ones of transpiration and yields brings out the fact that the approximately optimum moisture content here employed with the cultures of series B is correlated with maximum yields of tops and of roots, with high transpiration rates, and with water-requirement ratios which are intermediate in value between those of the other series (series A and C), having degrees of moisture in the sand cultures considerably below and above that of the optimum.

#### SUMMARY

This paper is a report of studies on the influence of different degrees of moisture in a solid substratum upon the physiological salt balance for young wheat plants and upon the relative plant-producing value of various salt proportions.

Three different degrees of moisture were maintained in sand cultures: 40 per cent, 60 per cent, and 80 per cent of the water-retaining capacity of the sand. Tests were made with 36 different sets of salt proportions of the three salts  $\text{KH}_2\text{PO}_4$ ,  $\text{Ca}(\text{NO}_3)_2$ , and  $\text{MgSO}_4$  in solutions (Shive's optimum 3-salt series) with each of the three different degrees of moisture. The solutions, all having an initial total osmotic concentration value of 1.75 atmospheres, were supplied to the sand cultures in such quantities as to produce the different degrees of moisture. All three of the different moisture-content series were conducted simultaneously and were then repeated. The culture solutions were renewed every third day. The cultures were weighed each day, and the water loss by transpiration was restored through the entire growth period of 28 days. The growth period was the same for the first and the repeated series.

The main results of this study may be summarized as follows:

(1) For the set of conditions under which these tests were made the physiological balance of the nutrient solutions producing the best yields of wheat tops and roots was not altered by variations in the moisture content of the solid substratum to which the solutions were applied. The physiological balance of salt proportions which was best with the lowest moisture content was the best also with the medium and the highest degree of moisture.

(2) A slight shifting of the physiological balance, as this affects the growth of plants, is indicated for the growth of 9 high-yielding cultures, as a whole, out of a series of 36, with each increase in the moisture

content of the cultures, from a position in the series characterized by lower partial concentration of  $\text{KH}_2\text{PO}_4$  to one of higher partial concentration of this salt and correspondingly lower ones of  $\text{Ca}(\text{NO}_3)_2$  and  $\text{MgSO}_4$ .

(3) Good physiological balance and optimum total concentration of a nutrient solution for plants is not alone sufficient to produce the best growth of which the solution is capable when it is diffused as a film on the particles of a solid substratum. An optimum degree of moisture, under such conditions, is essential to impart to the soil (sand) solution its maximum physiological value. The actual plant-producing value of any fertilizer treatment is thus largely determined by the moisture conditions of the substratum.

(4) The lowest degree of moisture here employed with sand cultures is correlated with low yields of tops and of roots, with the lowest transpiration rates, and with the lowest water requirement ratios. The highest moisture content of the cultures, on the other hand, is associated with low yields of tops and of roots, with high transpiration rates, and with the highest water requirement ratios. The medium degree of moisture, which is approximately optimum for the substratum here used, is correlated with the highest yields of tops and of roots, high transpiration rates, and medium water requirement ratios.

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## TREATMENT OF CEREAL SEEDS BY DRY HEAT

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### INTRODUCTION

In the investigation of possible control measures for certain seed-borne diseases of cereals which do not yield to the ordinary chemical and hot water seed treatments, the authors found dry heat to be particularly adaptable. The progress made with these seed treatments seems to warrant the publication of this preliminary paper, giving a brief review of the pertinent literature, as well as the methods employed and the results obtained by the writers to date.

### REVIEW OF THE LITERATURE

The early literature, as well as some of the more recent papers relative to heat treatments, represents chiefly the results obtained by plant physiologists who were studying the effect of high temperatures and drying on germinability of various seeds, including those of certain cereals.

Edwards and Colin (9),<sup>1</sup> in 1834, were the first to make important contributions on the subject.

Heiden (14, p. 30-37), in 1859, showed that barley germinated after being exposed for one hour to dry air at 90° C., while similar grains heated in water at 60° C. for the same period of time were killed.

Sachs (24), in 1865, showed that moistened seeds of rye, barley, corn, peas, and flax were killed at from 50° to 60° C., while those containing less moisture withstood 70° C. Length of exposure was not mentioned.

Just (17, 18), in 1875 and 1877, found that clover and other kinds of seeds heated in a saturated atmosphere at 50° C. for 48 hours, or at 75° C. for 1 hour, lost their viability, while similar seeds endured a dry heat of 120° C. for 1 hour.

Von Höhnelt (15), in 1877, working with the seeds of various plants, reported that most of them when dry were able to endure exposure to 110° C. for 60 minutes and that some were found viable even when exposed to 125° C. for 15 minutes.

<sup>1</sup> Reference is made by number (italic) to "Literature cited," p. 388-390.



Nobbe (23), in 1897, dried rye grain at 80° C. for several hours with very little effect upon subsequent germination. More severe exposure to dry heat was found to injure seriously the germinability of rye, while wheat and oats were killed at shorter exposures.

Jodin (16), 1899, reports that if seeds of peas and cress are dried at 60° C. for 24 hours they can endure dry heat at 98° C. for 6 hours without injury, while similar seeds heated in humid air at 40° C. for 20 hours lose their viability.

Dixon (7, 8), in 1901 and 1903, found that if various kinds of seeds were previously dried over sulphuric acid in a desiccator they could withstand exposure to 95° C. for several days without losing their viability. He reports that well-dried seed will endure even somewhat higher temperatures, that is, 110° to 120° C., without injury.

Schneider-Orelli (25, 26), 1909 and 1910, Neuburger (22), 1914, Waggoner (29), 1917, Harrington and Crocker (13), 1918, and others (5, 6, 11, 12), working with various seeds, have confirmed, in general, the results of former investigators—that various seeds are able to endure high temperatures, especially when their moisture content is low.

It was not until about 1900, however, that plant pathologists began to appreciate the possibility of using dry heat as a means of control for certain seed-borne diseases.

In 1908 there appeared almost simultaneously several papers on the subject of smut control by dry heat. Kühle (19) reported that the spores of stinking smut are killed when exposed to 65° C. for 12 minutes and that loose smut also can be controlled by dry heat. For barley he used temperatures up to 90° C. and for wheat up to 110° C. without destroying the viability of the seed. The barley treated in this way was free from loose smut, but the results with wheat were not so successful. While Kühle does not mention the length of time during which the seed was treated, it probably is the same as that reported the same year by Störmer (27), who worked with him. Störmer used samples which were first dried at low temperatures and then heated for 10 minutes at each of the following temperatures: 50°, 65°, 75°, and 90° C. for barley and 50° to 60°, 65°, 85°, and 100° to 110° C. for wheat. The samples were then cooled down to room temperature after each 10-minute heat treatment. The results showed a marked decrease of smut in barley and only a slight decrease of smut in wheat.

For several years following 1908, dry heat treatments were abandoned by plant pathologists for a modified heat treatment in which seeds were first soaked in water and then subjected to high temperatures. This method was used with some success by Appel (1), Appel and Riehm (2, 3, 4), Gisevius and Böhmer (10), Lang (20), Störmer (28), and Westerdijk (30) for smut control. For example, Appel and Riehm (2, 3) soaked smutted seeds of barley and wheat in water for 4 hours and then heated them at 55° to 60° C. for 20 to 30 minutes. These

exposures gave smut-free plants, but the 30-minute treatment greatly reduced the percentage of germination.

Naumov (21, p. 149-162, 175), in 1916, after having obtained negative results from all known seed treatments in attempts to control wheat scab (*Gibberella saubinetii* (Mont.) Sacc. and *Fusarium* spp.), reports that he was able to kill the infections on the seeds of cereals by dry heat. Wheat, barley, and oats were subjected to 60° C. and rye to 65° C. for periods ranging from 24 hours to 3 days. This treatment, according to Naumov, killed the fungus mycelium present in the interior of the kernels or at least weakened it greatly.

#### EXPERIMENTS

The writers first attempted to duplicate Naumov's treatments, and found his results difficult to verify. Wheat and barley thus treated retained their viability, but so did the fungi *Gibberella saubinetii* and the *Fusarium*, *Helminthosporium*, and *Alternaria* species infecting the kernels of these grains.

Following this, higher temperatures and longer exposures were tested with rather surprising results. Some wheat and barley kernels remained viable even after an exposure to 100° to 110° C. for as long as 45 hours. It was soon found possible by somewhat reducing this time to lessen the injury to the seed and yet kill the most persistent parasites. The barley used in these earlier experiments was Chevalier, a 2-row variety, abundantly infected with *Helminthosporium sativum* P. K. B. and also to some extent with *Gibberella saubinetii*. The kernels most badly infected with *H. sativum* are readily detected by the dark brown germ ends; hence, such kernels were selected for the experiments. This fungus has an added advantage for experimentation in that it sporulates freely on culture media and can be identified readily. Furthermore, *H. sativum* in the seed is more difficult to kill by the ordinary methods of seed disinfection than most other parasites known to the writers. On account of this resistance to seed treatments and because it is easily identified, *H. sativum* was chosen as the main index of efficiency of the dry-heat treatments tried. The wheat used in the earlier trials was a durum wheat, Kubanka (South Dakota 75), which was infected with *Gibberella*, *Fusarium*, *Helminthosporium*, and *Alternaria*.<sup>1</sup>

With the exception of one series these experiments were all made in a gas-heated sterilizing oven. While it required considerable attention throughout the duration of the treatments to keep the temperatures reasonably constant, this was accomplished by careful watching and the regulation of gas supply and ventilation as necessary.

<sup>1</sup> This was kindly furnished by Prof. Manley Champlin, of the South Dakota Agricultural Experiment Station, Brookings, S. Dak.

## EXPERIMENT 1

In experiment 1, small lots of infected kernels of the Kubanka durum wheat and Chevalier barley were selected. These were exposed to 100° to 110° C. in the gas oven for 15-hour and 30-hour periods. About 10 series of culture experiments were made on these treated seeds to compare them with the untreated. In each culture 10 kernels each of barley and wheat were placed on potato agar poured plates and incubated at room temperatures. Surface disinfection with mercuric chlorid solution (1:1,000), for 30 minutes was used on the untreated kernels in order to disinfect the surfaces. In practically all cases *Gibberella*, *Fusarium*, *Helminthosporium*, and *Alternaria* developed rather uniformly from the unheated kernels of wheat and barley, as well as from the kernels which were heated for 15 hours. From the kernels heated for 30 hours, however, only one wheat kernel yielded the fungus *Gibberella*, and one barley kernel yielded *Helminthosporium*. In both of these cases the fungus growth was very weak and remained so.

## EXPERIMENT 2

Following these promising leads various grains were treated in a large electrically heated drying oven at a temperature of about 100° C., in order to test the effect of 15-hour and 30-hour exposures on germinability. Following the treatment 100 kernels were counted out from each heated sample and the same number from its untreated control and sown in sand in the greenhouse. Table I gives the germination results for this experiment:

TABLE I.—Effect of dry-heat treatment on germination of seed

Kind of grain.	Variety.	Percentage of germination.		
		Not treated.	Exposed at about 100° C. for—	
			15 hours.	30 hours.
Barley	Hulless	94	72	71
Do.	Beldi	73	69	47
Do.	Manchuria (crop of 1917)	100	96	88
Do.	Manchuria (crop of 1912)	96	79	61
Wheat	Winter wheat	93	92	91
Do.	Turkey	96	80	78
Do.	Kharkov	99	97	81
Do.	Kanred	98	100	98
Do.	Russian	100	99	97
Do.	Marquis	100	93	94
Do.	Dawson Golden Chaff	93	73	62
Do.	Marquis	92	94	72
Rye	Winter rye	98	88	82
Oats	Wisconsin Pedigree No. 1	98	94	90
Do.	Wisconsin Pedigree No. 5	95	66	62
Do.	Sixty-day (South Dakota 165)	98	81	67
Do.	Swedish select (South Dakota 112)	90	90	85

The results from this experiment show convincingly that good dry seed of barley, wheat, oats, and rye is able to withstand surprisingly well the high temperature used, up to 30 hours. Previous tests had shown this time and temperature to be fatal to even the persistent parasites. The Beldi barley used in the foregoing experiment was moderately infected with *Helminthosporium sativum*. Good data on the effect of the treatment on this parasite were obtained in the germination boxes as follows: Nine of the 73 plants from the untreated control seed and 9 of the 69 plants from the seed treated for 15 hours developed typical primary lesions of *H. sativum* and showed marked basal browning, while none of the 47 plants from the seed treated 30 hours showed either primary lesions of any kind or any basal browning. All plants from the treated seed were a trifle slow in starting, but in the second-leaf stage they had overtaken or surpassed the others and continued to develop normally until taken out. Table II summarizes the results from this barley infected with *H. sativum*.

TABLE II.—Effect of dry-heat treatment in experiment 2 on germination and the development of *Helminthosporium sativum* in Beldi barley<sup>1</sup>

Treatment.	Number of kernels sown.	Number of kernels germinated.	Number of plants with primary leaf lesions.	Basal browning.
None.....	100	73	9	+
15 hours at 100° C.....	100	69	9	+
30 hours at 100° C.....	100	47	0	—

<sup>1</sup> Seed sown in sand Mar. 28. Observations made Apr. 9.

While this was a weak sample of barley, as shown by percentage of germination in untreated seed, the results as to the effect of the treatment on the disease are striking—the 30-hour treatment completely eliminated the disease.

#### EXPERIMENT 3

Two other series of treatments were then undertaken, and the seed treated was used for further germination and infection experiments and in field sowings. In these experiments only seeds that were known to be infected with various diseases were used. The third experiment was started April 22 and completed April 23. The gas oven was used, as in experiment 1, and was watched very carefully through the 30 hours. Observations of the temperature were made at least every half hour and during most of the time at shorter intervals. The temperature range was from 95° to 105° C., averaging about 100° throughout. Samples of about 1 pint each of four different seed lots were used for germination tests and field sowings. For the germination tests, 100 kernels were counted from each treated sample and a like number from each corresponding

untreated control. They were all sown in a thin layer of sterile sand over sterile garden soil in the greenhouse. Table III summarizes the results obtained.

TABLE III.—Effect of dry-heat treatment in experiment 3 on seed germination and the development of *Helminthosporium sativum* in barley

Kind of grain.	Variety.	Number of kernels germinated.		Infection.			
		Un-treated.	Heated at 100° C. for 30 hours.	Untreated.		Treated.	
				Number of plants with primary leaf lesions.	Basal browning.	Number of plants with primary leaf lesions.	Basal browning.
Barley.....	Chevalier.....	85	71	21	+	0	—
Do.....	Oberbrucker.....	94	85	5	+	0	—
Do.....	Manchuria.....	97	58	1	+	0	—
Do.....	Beldi.....	86	55	14	+	0	—

Table III shows two things: First, that the barley was not killed by the very severe treatment, in fact proved quite resistant; second, that while there were heavy infections of *Helminthosporium sativum* in the untreated seed lots, there was perfect control of the disease in those treated. These conditions are illustrated in general in Plates 48 and 49.

In Plate 48, A, the two groups represent all the 85 plants resulting from the greenhouse experiment on the 100 untreated kernels of Chevalier barley referred to in Table III. At the left are shown the 21 plants with distinct leaf lesions from attacks of *Helminthosporium sativum*, while in the larger group at the right are represented the remaining 64 plants from the untreated seed that did not show leaf lesions. That all the plants in both groups showed marked darkening of the kernels is evident, also that many showed markedly discolored roots. The dark color of these kernels and the root discolorations are shown more strikingly in Plate 49, A, on the 5 plants at left.

In Plate 48, B, are shown all the 71 plants resulting from parallel greenhouse experiments on the 100 treated seeds of Chevalier barley referred to in Table III. These were perfectly free from *Helminthosporium sativum* attacks. Their bases were usually clear and clean. The kernels were much lighter-colored than untreated ones (Pl. 48, A), and the roots also were free from discoloration. This is more clearly seen in Plate 49, A, where typical plants from both groups are represented. The 5 plants at the right are from treated seed, while the 5 at the left are from untreated seed.

Although the infections were less severe in the other varieties, the results agree in general with those brought out in detail for the Chevalier barley. That is, infections resulted from untreated seed, and perfectly clean plants

## EXPERIMENT 4

The fourth experiment was started April 25 and completed April 26, 1918, the seed being heated for 30 hours. The same gas oven was used as for the third experiment. It was watched in the same way and the temperature range held the same, 95° to 105° C., averaging about 100°. Seed samples of about 1 pint were again used, but in this fourth experiment wheat, oats, and rye were tested.

In Table IV are given the results of greenhouse germination tests on the untreated and treated seed of both the third and fourth experiments.

TABLE IV.—Effect of dry-heat treatment in experiments 3 and 4 on germination

Kind of grain.	Variety.	Percentage of germination.	
		Untreated.	Heated for 30 hours at 100° C.
Barley.....	Beldt.....	86	55
Do.....	Beldt <sup>1</sup> .....	53	63
Do.....	Chevalier.....	85	71
Do.....	Oderbrucker (a) <sup>2</sup> .....	95	64
Do.....	Oderbrucker (b).....	94	79
Do.....	Oderbrucker (c).....	100	31
Do.....	Oderbrucker (d).....	92	59
Do.....	Oderbrucker (e).....	95	66
Do.....	Oderbrucker (f).....	95	85
Do.....	Manchuria.....	97	58
Do.....	Manchuria (Wisconsin pedigree No. 9).....	97	83
Wheat.....	Kubanka (South Dakota No. 75).....	66	30
Do.....	Preston (South Dakota No. 67).....	95	53
Do.....	Kanred.....	100	98
Do.....	Kharkov.....	97	80
Rye.....	Winter rye.....	92	16
Oats.....	Mixed variety.....	96	65
Do.....	Swedish Select.....	95	57

<sup>1</sup> Seed hand-picked from plants distinctly infected with bacterial blight in the heads.

<sup>2</sup> Oderbrucker a, b, c, etc., represent samples of same variety but from different sources.

It is evident from Table IV, as from Table I, that seeds of these various cereals withstand this severe drying surprisingly well. While with certain samples the germination was cut down severely, as with rye and Preston wheat, it should be noted that both of these were rather shrunken, especially the Preston wheat. The Kubanka wheat was also a weak sample.

The seed of the wheat varieties, Kubanka (South Dakota No. 75) and Preston (South Dakota No. 67), used in experiment 4, carried a considerable amount of scab infection (*Gibberella saubinetii* and *Fusarium* spp.). The untreated grain from both samples when sown in the greenhouse for the germination test gave a considerable number of plants showing seedling infections. The bases of many plants were discolored, and some plants were killed even before reaching the surface (Pl. No. 10, 11, 12, 13).

These symptoms are typical of seedling infections from the scab organism. The seedlings from the wheat of both varieties treated by the dry-heat method were free from any indication of disease (Pl. 49, B, at right).

#### FIELD SOWINGS AND RESULTS

Field sowings were made of all the seed lots of the barley, wheat, rye, and oats treated in experiments 3 and 4, as listed in Table IV. The sowings were made in an isolated place on the university farm at Madison, Wis. Seed from each lot was sown in from one to five rows, each 150 feet long and about 12 inches apart. Care was taken throughout to avoid contamination of seed from any source. In order to prevent secondary infections from other fields no similar grains, not even the control seedlings, were grown within about  $\frac{3}{4}$  mile of this plot. The control seedlings of a complete parallel series of untreated seed lots were grown in another field of similar soil type, elevation, and exposure. The plants, both from the treated and untreated seed lots, developed normally; and good stands resulted except from the Preston and Kubanka wheats. These stands were thin.

While the results with regard to disease on plants from the dry-heat-treated seeds were in certain respects disappointing, yet in others they were rather encouraging, and in still others they were very satisfactory, as shown by the following brief account.

**BACTERIAL BLIGHT OF BARLEY.**—The bacterial blight of barley (*Bacterium translucens*, J. J. R.) was controlled perfectly by the dry-heat treatment as used, not even the slightest trace of this disease being noted in the plot from the treated seed, though the seed was known to be heavily infected. The corresponding plot from untreated seed, on the other hand, showed abundant infections with the disease.

The perfect control of the bacterial disease of barley is highly significant. The results were very definite and striking—perfect control in the treated plots and abundant disease in the untreated plots. Furthermore, there are indications that this bacterial organism of barley is more resistant in the seed than is that of bacterial disease of wheat known as "blackchaff." The above results would indicate the very strong likelihood that this dry-heat treatment will prove highly efficient in controlling the "blackchaff" of wheat. The data given above show definitely that wheat of good quality will stand the treatment.

**BACTERIAL BLIGHT OF OATS.**—The perfect control of the bacterial blight of oats (*Pseudomonas avenae*) was equally definite. It was the more striking because this disease was general throughout southern Wisconsin during the season of the experiment (1918). In fact, while numerous fields were examined, the only field of oats noted where this bacterial disease could not be found was the plot sown from dry-heat-treated seed. The untreated plot showed abundant infection with the

disease. Oats in good condition will withstand successfully the 30-hour treatment, but it is probable that even a less severe exposure will be found to control the organism effectively.

**WHEATSCAB.**—The Kubanka and Preston wheats used in experiment 4 were heavily infected with scab (*Gibberella saubinetii* and *Fusarium* spp.). The seedlings from the treated seed showed no attacks from this disease, while the ones in the control plot from the untreated seed showed numerous primary infections. Likewise later, when in head, the plants from the treated seed showed no scab in the head, while the wheat in the isolated control plot showed an abundance of such infections. These data, while yielding encouraging indications, of course point only to the possibility of eliminating seed infection.

**SPOTBLOTCH OF BARLEY.**—The Chevalier barley used in experiment 3 was heavily infected with spotblotch (*Helminthosporium sativum*), as illustrated in Plate 48, A. The thousands of plants in the plot from treated seed were carefully examined, and only four leaf lesions were noticed on the young seedlings. This would seem to indicate that spotblotch was not quite perfectly eliminated in the field, though from the greenhouse experiments previously mentioned its elimination might have been expected. The disease was present in considerable abundance in the control plot planted with untreated seed.

**NETBLOTCH AND STRIPE DISEASE OF BARLEY.**—Scattering primary infections of netblotch (*Helminthosporium teres*) on barley and several infections of stripe disease of barley (*H. gramineum*) were noted in the plots planted with the dry-heat-treated seed. An abundance of both these diseases occurred in the control plot planted with the untreated seed. The same was true of the *Helminthosporium* leafblotch of oats (*H. avenae-sativae*).

**SMUT.**—The percentage of loose smut infection in barley and oats was considerably diminished by the 30-hour heat treatment. In comparison with the numerous smutted heads in the control plots only a few appeared in the plots sown with treated seed.

Work is being continued on the problem.

#### SUMMARY

(1) The work here reported, while only a beginning, suggests promising possibilities.

(2) The data at hand indicate that the various cereals—barley, wheat, rye, and oats—especially when of good quality and well-dried, are able to withstand protracted exposures to dry heat at comparatively high temperatures.

(3) It is definitely shown that the seed infections from bacterial blight of barley (*Bacterium translucens*) and the bacterial blight of oats (*Pseudomonas avenae*) may both be eliminated by exposing the infected seed to dry heat at temperatures which leave the seed still viable.



(4) The results of these experiments indicate that a number of seed-borne fungous diseases, such as wheat scab (*Gibberella saubinetii* and *Fusarium* spp.), primary infections only, and spot blotch of barley (*Helminthosporium sativum*), are practically eliminated by the dry-heat treatment as used. Other diseases like net blotch (*H. teres*), stripe disease (*H. gramineum*) of barley, and *Helminthosporium* blotch of oats (*H. avenae-sativae*), as well as loose smut of barley and smuts of oats, are markedly reduced by the dry-heat treatment without materially injuring the germination of the seed.

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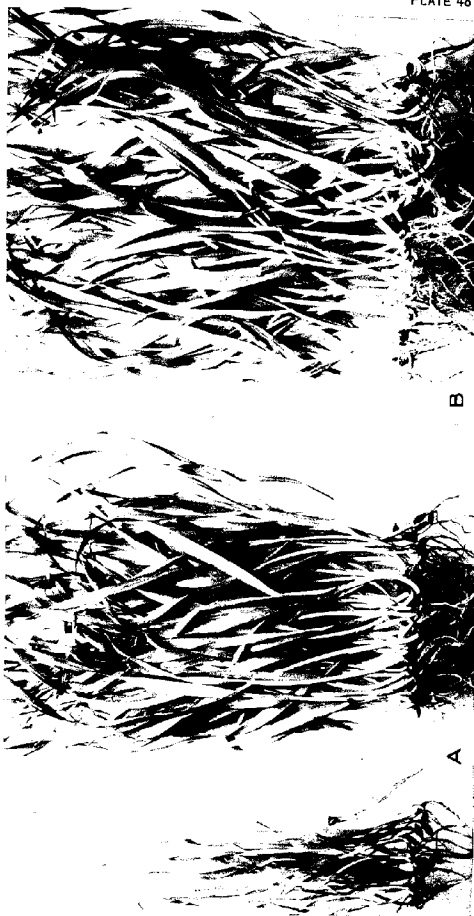
PLATE 48

Chevalier barley plants from untreated and treated seed referred to in Table III:

A.—Plants from untreated seed. At left are illustrated the 21 plants showing the most marked evidence of *Helminthosporium sativum* primary infection: leaf lesions, basal browning, and root rotting. At right are illustrated the other 64 plants showing less marked evidence of infection. Roots and kernels, however, show considerable discoloration.

B.—Plants from seed treated with dry heat. No diseased plants whatever; all 71 plants are clean and roots bright.

Approximately one-third natural size.



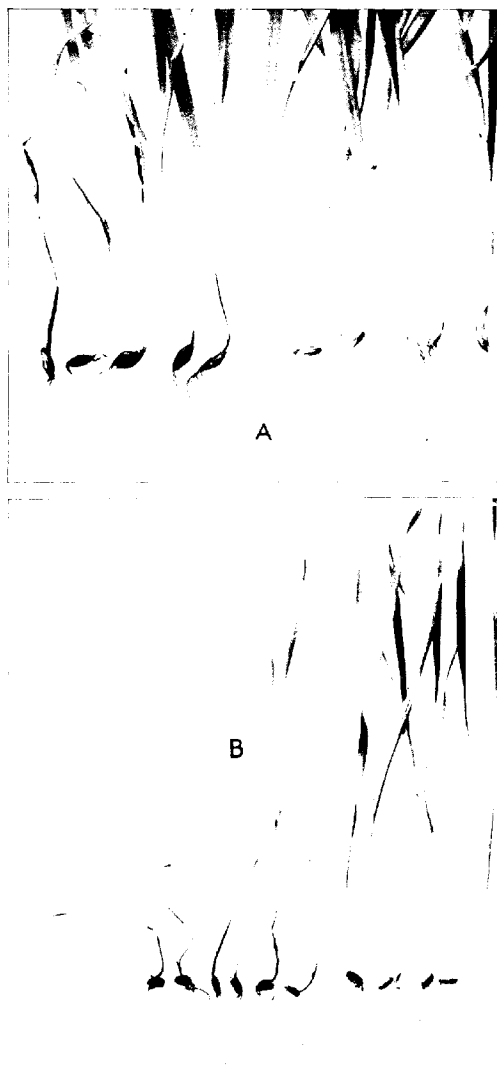


PLATE 49

A.—Basal portions of 10 representative Chevalier barley plants, from untreated and treated seed, selected from the lots illustrated in Plate 48. The 5 plants at left are from those shown in Plate 48, A, from untreated seed. Note the marked *Helmintosporium sativum* leaf lesions, basal browning, kernel discoloration, and root rotting. The 5 plants at right are from those shown in Plate 48, B, from the treated seed. Note the freedom from disease. There is no evidence of leaf lesions, basal browning, kernel discoloration, or root rotting; roots are clean and bright. All plants from seed treated by the dry-heat treatment were a trifle slower in germinating, but in the second leaf stage they had overtaken or surpassed those from untreated seed. This sturdier growth is evident in the illustration.

B.—Representative Kubanka wheat seedlings from untreated seed (6 plants at left) and treated seed (5 plants at right) referred to on pp. 385-386. The 6 plants at the left show characteristic seedling injury from wheatscab organisms. All 6 of these plants had discolored kernels, rotted bases, and rotted proximal portions of roots. They were also much weaker than those from the treated seed. The first 2 plants on the left were killed after they reached the surface of the ground; the third plant was killed before reaching the surface of the ground. Typical *Gibberella perithecia* developed on this dead plant under the soil and at the surface. The 5 plants at the right represent the disease-free condition of plants from treated seed from the same seed lot. This seed had been exposed to dry heat at about 100° C. for 30 hours. The plants are free from any evidence of disease, the bases, kernels, and roots all being clean. Plants from treated seed are also much sturdier than those from untreated seed.

Approximately one-half natural size.





## MEAT SCRAPS VERSUS SOYBEAN PROTEINS AS A SUPPLEMENT TO CORN FOR GROWING CHICKS

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The proteins found in natural feed stuffs vary greatly in their nutritive value as well as in their solubility and in the proportions of the different amino acids which they are capable of yielding. The cereal grains contain most of the important amino acids but apparently in many cases in proportions unsuitable to promote growth and development. McCollum and his coworkers<sup>1</sup> have shown that the cereal grains, although they have a low biological value as compared to milk, have a remarkable value as supplementary sources of amino acids for certain vegetable proteins. It is thought that the value of corn proteins in producing growth has been somewhat underestimated. This may be due to the fact that one of the proteins (zein) which is usually present in a considerable amount, lacks two important amino acids (lysin and tryptophane), and the young animal fed on corn is incapable of appreciable growth on the only proteins remaining (glutelin, globulins, and albumins). Data obtained by R. H. Carr and coworkers<sup>2</sup> would seem to indicate that the amount of zein in mature corn is somewhat overestimated, and hence that the other proteins present were probably largely responsible for the consistent growth which was secured with chicks fed on corn containing less than 10 per cent proteins fortified with ash and with fat-soluble vitamins. The proteins of meats are credited with having a high value in producing growth, but their relative efficiency as compared with vegetable protein is not so well understood. The proteins of soybean are usually considered of excellent quality,<sup>3</sup> but their biological value is thought to be of the same order as that of corn and oats.

Most of the work done so far in measuring the biological value of proteins from various sources has been conducted on the rat or the pig, because these animals have many points of advantage for laboratory investigations. The growing chick<sup>4</sup> has been used by some investigators,

<sup>1</sup> McCOLLUM, E. V., SIMMONDS, N. and PARSONS, H. T. SUPPLEMENTARY RELATIONSHIPS BETWEEN THE PROTEINS OF CERTAIN SEEDS. *In* Jour. Biol. Chem., v. 37, no. 1, p. 155-178, 7 charts. 1918. Bibliog. Suppl., p. 177-178.

<sup>2</sup> SCHITZER, George, CARR, R. H., and EPPLE, W. F. SOFT CORN—ITS CHEMICAL COMPOSITION AND NITROGEN DISTRIBUTION. *In* Jour. Amer. Chem. Soc., v. 41, no. 8, p. 1311-1321. 1919.

<sup>3</sup> DANIELS, Amy L., and NICHOLS, Nell B. THE NUTRITIVE VALUE OF THE SOYBEAN. *In* Jour. Biol. Chem., v. 34, no. 1, p. 91-103. 1917.

notably Drummond,<sup>1</sup> who reports much difficulty in securing growth of the chicks in confinement. The chicks were troubled with "leg weakness" and "ruffled appearance," both of which defects were attributed to the lack of exercise in the open air. Osborne and Mendel<sup>2</sup> report partial success in raising chickens in confinement. Although they also report much difficulty with the chickens on account of "leg weakness," they were successful in raising several birds which seemed to develop quite normally. There are good reasons why it is desirable to use growing chicks in the laboratory to test the biological value of feeds. They represent an entirely different species from that of the rat or the pig, and it is hardly logical to translate the results secured on those animals to a species lower than mammals in the evolutionary scale. The ease with which it is possible to hatch eggs in an incubator and the rapid rate at which chicks grow and reach maturity, as well as the comparatively

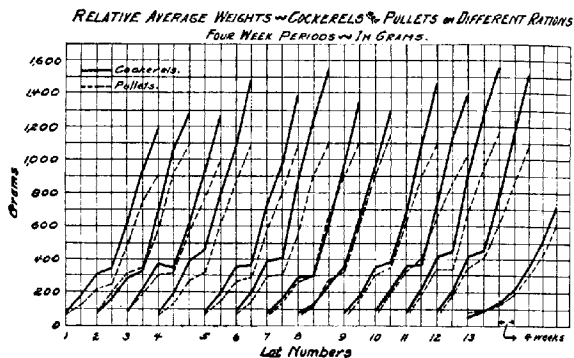


FIG. 1.—Graph showing the rate of growth of males and females in all lots.

small amount of feed required, are points in their favor for investigative work. The problem of securing normal growth in confinement has presented the greatest drawback to the successful use of chicks for this purpose. In the work here reported the writers had little difficulty with the disease known as "leg weakness" in the chicks, or with the other troubles usually experienced when rearing chicks in confinement, as reported in the literature. However, serious trouble was experienced from the eighth to twelfth week, when the chicks were developing a heavy growth of plumage. During this time 64 per cent of the total mortality occurred. The immediate cause of mortality was apparently excessive intestinal fermentation. After this critical stage was passed no further

<sup>1</sup> DRUMMOND, Jack Cecil. OBSERVATIONS UPON THE GROWTH OF YOUNG CHICKENS UNDER LABORATORY CONDITIONS. *In* *Biochem. Jour.*, v. 10, no. 1, p. 77-88, 1 pl. 1916.

<sup>2</sup> OSBORNE, Thomas B., and MENDEL, Lafayette B. THE GROWTH OF CHICKENS IN CONFINEMENT. *In* *Jour. Biol. Chem.*, v. 33, no. 3, p. 437-438, pl. 4-6. 1918.

trouble was experienced, and practically all the remaining chicks developed in a normal way. See figure 1.

#### OBJECT OF EXPERIMENT

The object of this experiment was to determine the value of corn protein in the growth of chicks when the proteins were fortified with sufficient ash and with fat-soluble vitamins, as compared with their value when supplemented by varying amounts of proteins derived from meat scraps or soybean meal or from these proteins in combination.

#### PLAN OF PROCEDURE

The stock used was 210-day-old White Leghorn chicks from the Purdue Poultry Farm. The chicks were hatched May 6 and divided into 14 lots of 15 chicks each. The initial individual weights of all the chicks were recorded on the sixth day, when all lots were given their respective rations tabulated in Table I. During the first 6 days all lots were given only granulated corn, grit, and water. Individual weights of the chicks were taken every 14 days after the initial weights were recorded. Each ration was "weighed back" on the same day the chicks were weighed in order to obtain the feed consumption for each period of 14 days. The growth period of the experiment was closed at the end of 26 weeks, but the pullets were kept for a longer time to note results of egg production.

The method of care and management of the chicks was that which is generally advocated for the successful rearing of chicks. Since the chicks were confined in pens 4 by 6 feet during the entire experiment, special effort was made to feed them so that they would be active as much of the time as possible and thereby avoid the evils of overfeeding.

Table I gives the rations, in part by weight, received by each lot.

In addition to these rations, each lot received water, grit, oyster shells, and about 75 gm. of the tops of sprouted oats. Oat straw was used for scratching litter. Lot 13, used as a control pen, received the basal ration only. The ash mixture was omitted from the ration of lot 2a as a control on the ash; otherwise this lot received the same ration as lot 2. Since there was no appreciable difference in the results obtained from these two lots, no further reference will be made to lot 2a. In each case the amount of protein concentrate (meat scraps or soybean meal) added to the basal ration is based upon a definite amount of crude protein from that source as shown in Table I. The amount of protein concentrate used depended upon its content of crude protein as determined by chemical analysis. Chemical analyses were made also of the other feeds which entered into the rations. The same feeds were used during the entire experiment.

TABLE I.—*Ration supplied to growing chicks during 26 weeks of experiment*  
 [Expressed in parts by weight]

Lot No.	Basal ration.						Meat scraps.	Soy-bean meal.	Nutri- tive ratio.
	Grain.		Mash.						
	Ground corn.	Corn meal.	Corn bran.	Ash. <sup>a</sup>	Char- coal.	Crude protein.			
1.....	50	35	15	3	3	5	8.86	.....	1: 5.9
2.....	50	35	15	3	3	10	17.7	.....	1: 4.4
2a.....	50	35	15	0	3	10	17.7	.....	1: 4.4
3.....	50	35	15	3	3	15	26.6	.....	1: 3.5
4.....	50	35	15	3	3	20	25.4	.....	1: 2.9
5.....	50	35	15	3	3	5	.....	10.9	1: 6.2
6.....	50	35	15	3	3	10	.....	21.8	1: 4.8
7.....	50	35	15	3	3	15	.....	32.7	1: 3.9
8.....	50	35	15	3	3	20	.....	43.6	1: 3.4
9.....	50	35	15	3	3	5	4.4	54.5	1: 6
10.....	50	35	15	3	3	10	8.86	10.9	1: 4.5
11.....	50	35	15	3	3	15	13.3	16.4	1: 3.7
12.....	50	35	15	3	3	20	17.7	21.8	1: 3.2
13.....	50	35	15	3	3	.....	.....	.....	1: 9

<sup>a</sup> The ash mixture used in the above rations was composed of the following ingredients, expressed in parts:

Bone ash.....	50
Calcium carbonate.....	50
Sodium chloride.....	14
Dipotassium phosphate.....	13
Calcium lactate.....	10
Magnesium sulphate.....	3
Sulphur.....	3
Iron sulphate.....	1

100

Table II shows the average total amount of feed and its protein content which was consumed in 13 periods of 14 days each and the ratio of the protein fed to the gain.

TABLE II.—*Ratio between average feed consumed and average gain in weight in 13 periods of 14 days each*

BASAL RATION PLUS MEAT SCRAPS

Lot No.	Feed consumed.	Protein in feed.	Protein consumed.	Average gain per 14-day period.	Ratio of protein in feed to gain.
	Gm.	Per cent.	Gm.	Gm.	
1.....	535	12.44	66.34	87.00	1: 1.31
2.....	598	15.88	94.96	85.3	1: 0.89
3.....	509	18.60	94.67	80.60	1: 0.85
4.....	546	20.9	114.10	87.20	1: 0.76
Average.....	547	.....	92.52	84.5	1: 0.95

TABLE II. *Ratio between average feed consumed and average gain in weight in 13 periods of 14 days each—Continued*

## BASAL RATION PLUS SOYBEAN MEAL

Lot. No.	Feed consumed.	Protein in feed.	Protein consumed.	Average gain per 14-day period.	Ratio of protein in feed to gain.
	Gm.	Per cent.	Gm.	Gm.	
5.....	530	12.53	66.40	89.80	1:1.35
6.....	631	15.36	96.92	104.30	1:1.08
7.....	533	17.7	94.34	97.30	1:1.03
8.....	554	19.8	109.10	90.2	1:1.83
Average.....	561		91.69	95.30	1:1.07

## BASAL RATIO PLUS COMBINATION OF MEAT SCRAPS AND SOYBEAN MEAL

9.....	523	12.64	66.10	90.9	1:1.05
10.....	615	15.6	95.04	101.20	1:1.17
11.....	620	18.11	113.85	99.80	0.88
12.....	582	20.31	118.15	98.00	1:0.83
Average.....	587		94.60	97.48	1:1.03

## BASAL RATION ONLY

13.....	365	9.12	33.29	47.92	1:1.44
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In this table the growth-promoting value of the protein is expressed numerically as suggested by Osborne and Mendel.<sup>1</sup> The table shows also that protein from meat scraps alone as a supplement to the basal ration in any amount was not equal to that from soybeans or from the combination of the two. Among the different lots (except the control lot 13) the average amount of feed consumed during the 13 periods of 14 days each did not vary greatly, ranging from 509 gm. for lot 2 to 631 gm. for lot 6. Hence it is quite important, from the standpoint of economy in feeding, to combine the amount and kind of protein with the basal ration in the proportion which produces the best growth. In this instance the best results were shown by lot 6, which received 10 parts of protein from soybean meal. Next in order are lots 10, 11, and 12, which received 10, 15, and 20 parts protein, equally from meat scraps and soybean meal. Following these are the lots receiving protein from meat scraps. Plate 50 shows a cockerel and a pullet from lot 13, a cockerel from lot 10, and a pullet from lot 6.

<sup>1</sup>OSBORNE, Thomas B., MENDEL, Lafayette B., and PERRY, Edna L. A METHOD OF EXPRESSING NUMERICALLY THE GROWTH-PROMOTING VALUE OF PROTEINS. *1st Jour. Biol. Chem.*, v. 37, no. 2, p. 223-229, 1918.

The aim was to supply a sufficient amount of ash to each ration to meet all mineral requirements and so have but one variable protein running through the whole series of rations. The addition of ash to the rations containing meat scraps was probably unnecessary, but in order to secure uniformity the same amount was added to all.

Table III gives the percentage of nitrogen and ash in the feces at different periods of the experiment.

TABLE III.—Distribution of feces nitrogen and ash at different periods

## BASAL RATIO PLUS MEAT SCRAPS

Lot No.	Nitrogen and ash content of feces from chicks 4 weeks old.				Nitrogen and ash content of feces from chicks 20 weeks old.				Average total nitrogen in feces. <sup>a</sup>
	Total nitrogen.	Total nitrogen soluble in N/10 hydrochloric acid.	Total nitrogen insoluble in N/10 hydrochloric acid.	Ash.	Total nitrogen.	Total nitrogen soluble in N/10 hydrochloric acid.	Total nitrogen insoluble in N/10 hydrochloric acid.	Ash.	
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	
1.....	2.63	35.0	65.0	31.04	3.25	37.6	62.2	24.60	2.73
2.....	3.03	35.8	64.2	35.91	3.76	20.1	70.9	27.76	3.30
3.....	2.76	31.8	68.2	40.18	3.45	18.5	81.5	27.26	3.35
4.....	4.21	35.7	64.3	32.99	6.23	15.7	84.3	20.83	4.44
Average..	3.31				4.17				3.32

## BASAL RATIO PLUS SOYBEAN MEAL

5.....	2.46	35.1	64.9	28.94	3.33	25.2	76.8	18.44	2.74
6.....	2.72	35.9	64.1	28.49	4.50	19.1	80.9	20.02	3.02
7.....	3.05	29.4	73.6	31.49	5.61	16.0	83.1	17.77	4.49
8.....	2.63	25.1	74.9	44.55	7.23	10.8	89.2	19.00	4.42
Average..	2.71				5.19				3.82

## BASAL RATIO PLUS COMBINATION OF MEAT SCRAPS AND SOYBEAN MEAL

9.....	2.63	31.0	69.0	25.07	3.95	20.6	79.4	17.12	2.91
10.....	2.59	37.1	61.9	32.89	4.87	18.1	81.9	28.50	3.52
11.....	3.02	31.6	68.4	36.84	5.45	24.5	75.5	17.72	4.00
12.....	4.00	32.5	67.5	36.30	7.63	14.6	85.4	22.26	4.02
Average..	3.07				5.48				3.81

## BASAL RATIO ONLY

13.....	1.91	34.4	65.6	31.75	2.70	26.9	73.1	15.36	2.24
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<sup>a</sup> Average total nitrogen is the average for the 4-, 8-, 16-, and 20-week periods.

The table may be summarized as follows:

Lot No.	Ration.	Average total feces nitrogen.	Increase in waste.
		Per cent.	Per cent.
13.....	Basal.....	2.24	.....
1, 5, 9.....	Basal+5 parts protein.....	2.79	24.5
2, 6, 10.....	Basal+10 parts protein.....	3.57	59.4
3, 7, 11.....	Basal+15 parts protein.....	3.95	76.3
4, 8, 12.....	Basal+20 parts protein.....	4.59	105.0

The nitrogen soluble in *N/10* hydrochloric acid was considered to be ammonia, urea, and amino acid nitrogen. The nitrogen insoluble in *N/10* hydrochloric acid was considered to be uric acid and residual nitrogen.

The data in Table III indicate that in all lots receiving 5 parts of protein in addition to the basal ration, the excreta contained an average of 2.79 per cent nitrogen; in lots receiving 10 parts protein, they contained an average of 3.57 per cent; in lots receiving 15 parts, they contained an average of 3.95 per cent; in lots receiving 20 parts protein, they contained an average of 4.59 per cent; whereas in the lot receiving the basal ration only, they contained an average of 2.24 per cent nitrogen. This last figure was taken as maintenance nitrogen excretion. Since the feces in lots receiving 5 parts protein in addition to maintenance contained 2.79 per cent nitrogen, it was computed that the waste in excretion was 24.5 per cent greater than when the basal ration alone was fed. In the same manner 59.4 per cent more feces nitrogen was obtained for lots receiving 10 parts protein, 76.3 per cent more for lots receiving 15 parts, and 105 per cent more for lots receiving 20 parts. In brief, the greatest gain in weight was made with the least necessary nitrogen loss in feces when the basal ration was supplemented with 10 parts of protein.

It will be noted in Table III that the ash content of the feces collected when chicks were 4 weeks old and growth was most rapid was much greater for all lots than that of the samples collected when the chicks were 20 weeks old and growth was less rapid and maintenance requirements were greater. The excretion of nitrogen was very constant for all lots receiving the same amount of protein; and since the protein consumed increased by 5 parts in four successive rations, the average increases over control lot 13 were 2.79, 3.57, 3.95, and 4.59 per cent, respectively, for each addition of 5 parts of protein to the basal ration. Thus it would appear that there was no economy in nitrogen excretion at the point where the gain was most efficient (the addition of 10 parts protein), though such an economy might have been expected. Table III also shows that a 2-gm. sample of feces of any nitrogen content contained nearly the same weight of *N/10* acid-soluble nitrogen (ammonia,



urea, and amino acids) regardless of the total weight of nitrogen in the sample, and that only the uric acid, etc., increased in amount as more protein was fed.

#### SUMMARY

In conclusion, it would seem that it is possible to secure nearly normal growth of chicks when raising them in confinement, and that this method has many points of advantage as a means of measuring the biological value of feeds for chickens.

These results indicate that there is a wide range in the amount of protein which may be fed with little difference in results except in economy in feed consumption.

When the basal ration was supplemented with varying amounts of protein from meat scraps, soybean meal, or combination of the two, it is shown that an addition of 10 parts of protein from soybean meal gave the best growth. The next best gains came from 10, 15, and 20 parts of protein from the combination of soybean meal and meat scraps. All the meat scraps rations were found to be somewhat inferior to those of the soybean meal or the combination.

The amount of nitrogen present in the feces as ammonia, urea, or amino acids (soluble in *N/10* hydrochloric acid) was nearly constant regardless of the total nitrogen present in any sample, the remainder of the nitrogen present being due largely to the uric acid. The amount of excreted nitrogen was dependent on the amount of the protein consumed and increased proportionately.

The data which have been presented tend to show that chicks are capable of greater utilization of soybean meal protein than are mammals, with which nearly all previous nutritional work has been done.



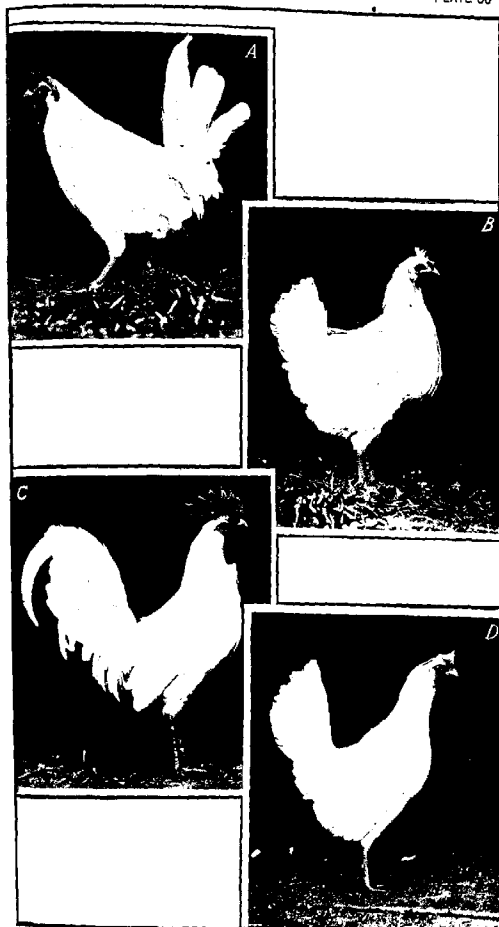
PLATE 50

A.—Cockerel No. 43, lot 13, fed on basal ration only. Weight, 710 gm. In perfect health but slow of growth.

B.—Pullet No. 44, lot 13, fed on basal ration only. Weight, 705 gm. Feathers not mature. Bird growing slowly but naturally, and gradually approaching maturity.

C.—Cockerel No. 54, lot 10, fed on basal ration plus five parts meat scraps protein and 5 parts soybean meal protein. Weight, 1,620 gm. Bird vigorous and normal in every respect.

D.—Pullet No. 82, lot 6, fed on basal ration plus 10 parts soybean meal protein. Weight, 1,295 gm. Bird vigorous, laying, and normal in every way.





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